PCDD/Fs AND DL-PCBs IN MEAT AND LIVER TISSUES OBTAINED FROM CHICKENS FED WITH EXPERIMENTAL SPIKED FEEDS CONTAINING LEVELS OF THESE POLLUTANTS CLOSE TO THE MAXIMUM ESTABLISHED AT THE EU DIRECTIVE

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Introduction

The fact that the main route of human exposure to polychlorodibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), and also to "dioxin-like" polychlorinated biphenyls (DL-PCBs), take place through food compsumtion is nowadays well documented and accepted[1]. Taken into account that among the different food products, those with an animal origin contribute largely to the exposure to this type of pollutants, several measures have been undertaken in order to decrease PCDD/F and DL-PCB levels in meat and other related products obtained from farmed animals. For instance, the European Commission has established maximum levels for these contaminats in feeds and in a wide range of raw materials for feedstuff production[2].

The FEEDING FATS SAFETY project (6th EC Framework Programme) deals with the control of animal nutrition in order to preserve animal health and to produce safe and good quality meat products at the same time. More specifically, the objective of the project is related to the use of fats (by-products or co-products derived from the food chain) as ingredients of the feeds and the corresponding effects that this practice would have on animal production. In this sense, a first study was carried out in which separate groups of two different types of animals, chickens and rabbits, were fed with three different experimental feeds containing certain percentages of two selected fish oils. At the end of the experiment, the levels of PCDD/Fs and DL-PCBs were determined in meat samples obtained from these animals[3].

The work presented in here was done as a continuation of the study previously mentioned. In the present case, the idea was also to prepare several experimental feeds, but spiked with different given amounts of PCDD/Fs and DL-PCBs by adding commercial standard mixtures of these compounds. The aim was to evaluate how the presence in the feed of high levels of PCDD/Fs and DL-PCBs, close to or above the maximum set at the EU Directive 2006/13/EC, would affect the levels of these pollutants present in animals feeding those feeds. In particular, the experimental design included the raise of four separate groups of chickens using four different feeds with increasing levels of PCDD/Fs and DL-PCBs. The spiked levels were: 0 (non-spiked feed), half of the maximum level, the maximum and 2-fold the maximum level.

Materials and Methods

Four treatments were designed to be included in the present study, accordingly four experimental feeds were prepared, a different one for each one of the treatments. The feed for Treatment 1 was a common feed for chickens, mostly composed of a mixture of raw materials of vegetable origin. A 6% vegetable oil was also included as an ingredient in this feed. Similarly, the rest of the feeds were prepared by adding to the former mixture base the same percentage of oil, but spiked with known amounts of EPA-1613PAR and WP-STK standard solutions (Wellington Laboratories Inc., Guelph, Canada), which contain native PCDD/Fs and DL-PCBs, respectively, in order to have a certain level of contamination. In this sense, the theoretical values expected in the feeds after their preparation were:

Treatment 1: Background level (non-spiked feed)
Treatment 2: 0.38 ng WHO- TEQ/kg feed (PCDD/Fs) and 0.38 ng WHO-TEQ/kg feed (DL-PCBs), which is approximately half of the maximum levels established at the EU Directive 2006/13/EC for PCDD/Fs and the sum of PCDD/Fs+DL-PCBs.

Treatment 3: 0.75 ng WHO- TEQ/kg feed (PCDD/Fs) and 0.75 ng WHO-TEQ/kg feed (DL-PCBs), the maximum levels established at the EU Directive 2006/13/EC for PCDD/Fs and the sum of PCDD/Fs+DL-PCBs.

Treatment 4: 1,5 ng WHO- TEQ/kg feed (PCDD/Fs) and 1.5 ng WHO-TEQ/kg feed (DL-PCBs), 2 times the maximum levels established at the EU Directive 2006/13/EC for PCDD/Fs and the sum of PCDD/Fs+DL-PCBs.

Groups of 4 broilers (7 days old) were fed for 33 days with one of the four above mentioned feeds. Each one of these separate groups of 4 animals constitutes a replicate. A total of four replicates were considered for each of the treatments. At the end of the experimental period, the animals were slaughtered and two different types of tissues were collected for separate analysis: meat and liver. Meat samples were obtained from the legs of the animals and, in this case, the skin was also included. Samples of both tissues, meat and liver, were ground in order to homogenize them and they were stored in plastic bags.

Meat and liver samples, as well as a part of the four different feeds, were received in the Dioxin Laboratory of the IIQAB-CSIC for PCDD/F and DL-PCB analysis. First, the tissue samples were freeze-dried and re-homogenized again as a pretreatment step. Then, samples were extracted in a Soxhlet for ~24h with toluene:cyclohexane (1:1) after being spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS) and $^{13}C_{12}$-DL-PCBs, (WP-LCS) both from Wellington Laboratories Inc. (Guelph, Canada). Next, the extracts were rotary evaporated and kept in the oven overnight (105 ºC) in order to eliminate the solvents prior to gravimetrical fat determination. Afterwards, fat residues were dissolved again in *n*-hexane. Organic components, fat and other interfering substances were removed by treating the *n*-hexane extracts with sulphuric acid. The extracts were then rotary concentrated and filtered prior to the next clean-up step. Further sample purification and instrumental analysis by high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) are described elsewhere[4].

Results and Discussion

The concentrations of the individual toxic PCDD/F and DL-PCB congeners were determined in meat and liver samples from chickens corresponding to the four different treatments previously described. The feeds used in each one of the treatments were also analyzed in order to know the background PCDD/F and DL-PCB levels in the non-spiked feed and to check that the spiked concentrations in the other feeds were in good agreement with the theoretical expected values. In general, the animals that fed the non-spiked feed (Treatment 1) showed low levels of these contaminants in both types of tissues. On the contrary, significant higher values of PCDD/Fs and DL-PCBs were found in the tissue samples obtained from chickens belonging to the rest of the treatments (Treatments 2, 3 and 4).

The non-spiked feed had a total of 0.06 pg WHO-TEQ/g of PCDD/Fs and 0.09 pg WHO-TEQ/g when DL-PCBS were also included. Both levels are clearly below the maximum established at the EU Directive for this kind of matrix[2]. Meat samples from animals that were fed with this feed showed an upperbound mean value for PCDD/Fs of 0.21 pg WHO-TEQ/g fat (n=4), with some congeners being below the detection limit of the method. The DL-PCB level was also low and contributed in about a 28 % to the total WHO-TEQ of the meat samples (Mean value of 0.29 pg WHO-$TEQ_{PCDD/Fs+DL-PCBs}$/ g fat (n=4)). In the case of the liver tissue low levels of these two families of pollutants were also observed, however they were 3 to 5 times higher for PCDD/Fs and DL-PCBs, respectively, than those found in the meat, expressed in lipid weight.

Conversely, as it can be seen from Table 1, chickens feeding the 3 different spiked feeds clearly showed higher levels of PCDD/Fs and DL-PCBs in comparison with those found in the animals from Treatment 1. In particular, meat samples from chickens from all these 3 treatments showed concentrations of PCDD/Fs and PCDD/Fs + DL-PCBs, in terms of pg WHO-TEQ/g fat, that exceed the limit values set at the EU Regulation[5]. In addition, the samples of liver tissue also showed levels of PCDD/Fs and DL-PCBs relatively high (Table 1) taking into account the concentration previously found in the liver samples from the Treatment 1 chickens, with several values also exceeding the maximum established at the EU Regulation 199/2006. It must be pointed out that, in the case of the meat samples, the high levels observed could be explained to a certain extend due to the inclusion

of the chicken skin for the analysis, which increased the percentage of fat of the samples up to approximately a 14%. The possibility that a different degree of bioaccumulation could take place in the meat and in the skin should be taken into account in order to correctly understand the levels of PCDD/Fs and DL-PCBs observed in this particular experiment.

In general, for a given treatment, the concentration of PCDD/Fs in the liver tissue of the chickens, expressed in pg WHO-TEQ/g fat, was about 2-3 times the level found in the meat+skin samples obtained from the same animals. Similarly, when the sum of PCDD/Fs and DL-PCBs is considered, the levels in liver are higher than in meat. This fact correlates well with which is already included in the EU Regulation 199/2006, that allows higher maximum levels in liver than in meat and meat products[5].

Congener distribution of PCDD/Fs, in pg/g fat, in the two types of tissue samples from the 3 different spiked treatments resembled the profile observed in the corresponding feeds, as it can be seen from Figure 1. This is in good agreement with the results previously obtained in some other experiments also carried out in the framework of the FEEDING FATS SAFETY project[3]. Chickens were able to bioaccumulate all the toxic PCDD/F and DL-PCB congeners, both in the meat and in the liver tissues. However, as it was also observed in the previous experiments, it seems that the percentage of the lowest chlorinated PCDD/Fs is higher in the meat and the liver samples compared to what is present in the feed and, on the contrary, the highest chlorinated congeners of PCDD/Fs represent a higher percentage in the feed samples.

In summary, the main conclusion from the present study is that meat and other products (i.e. liver) from chickens fed a feedstuff containing low levels of PCDD/Fs and DL-PCBs did not show a significant contamination related to these two families of compounds. The PCDD/F and DL-PCB concentrations in this kind of matrices, in terms of pg WHO-TEQ/g fat, were considerably lower than the maximum levels established at the EU Regulation 199/2006. In contrast, the same products obtained from animals that were fed contaminated feeds, in the present work spiked experimental feeds, showed levels of these pollutants that exceed the maximum allowed. In addition, this fact was particularly noticeable in Treatment 2 since, in this case, even when the values in the corresponding feed were high but just about half of the maximum limit set for compound feedstuff, the levels of PCDD/Fs and the sum of PCDD/Fs and DL-PCBs in the meat samples, as well as the level of PCDD/Fs in the liver tissue, were above the maximum allowed by the EU Regulation 199/2006.

Table 1. Levels of PCDD/Fs and PCDD/Fs+DL-PCBs, expressed in pg WHO-TEQ/g fat (upperbound values), in meat and liver samples from chickens from the four different treatments. In **bold,** values that exceed the maximum established at the EU Regulation 199/2006.

pg WHO-TEQ/g fat "upperbound" n=4	Chicken meat			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
PCDD/Fs	0.21	**3.16**	**6.50**	**12.57**
Sum (PCDD/Fs + DL-PCBs)	0.29	**6.79**	**13.80**	**27.02**
pg WHO-TEQ/g fat "upperbound" n=4	Chicken liver			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
PCDD/Fs	0.72	**7.24**	**12.99**	**26.53**
Sum (PCDD/Fs + DL-PCBs)	1.13	10.64	**19.24**	**39.14**

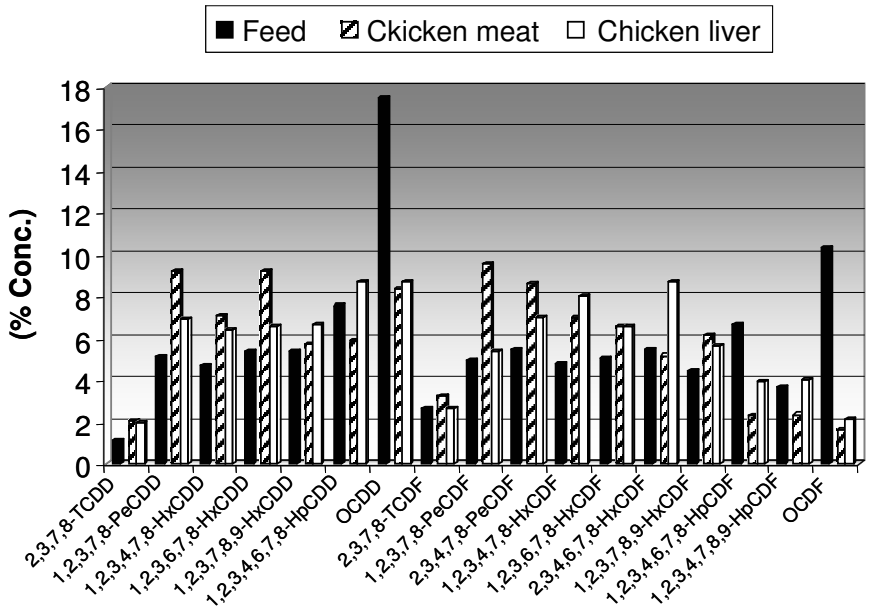

Figure 1. Comparison of PCDD/F profiles of feed, chicken meat and chicken liver samples from Treatment 2.

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