ASSESEMENT OF DIOXIN CONTAMINATION IN POULTRY (LIVER AND ADIPOSE TISSUE) FROM ROMANIA USING THE DR-CALUX BIOASSAY

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Introduction

Foodstuffs from animal origin, including poultry products, and edible offal such as liver, are potentially an important source of dioxins exposure for the consumer.

Analyzing the data extracted from the «Comprehensive European Food Consumption Database; Concise Data Base summary statistics- Total Population », it can be seen that the consumption of edible offal, including poultry liver, is between 1g/day in Ireland and 26,1g/day in Poland, with an average of 7.12 g/day for the European Union, considering the countries that participated to the survey[1].

Cell-based assays, such as the DR-CALUX, are the most used screening methods for dioxins, due to their capacity to analyze a large number of samples and lower costs comparing to MS-based methods[2]. This method is sensitive to all dioxin-like compounds that activate the Ah receptor.

Dioxin-like compounds bind to aryl hydrocarbon receptors (AhR) which are proteins largely present in liver. The major actions of dioxins are considered to be mediated by binding to the aryl hydrocarbon receptor (AhR) and subsequent transcription of several genes such as metabolizing cytochrome p 450 enzymes[3, 4].

In poultry, the toxicokinetic study made by Pirard and co-workers[5] concluded that abdominal fat and liver of chickens are the major storage sites for dioxins and liver retains a large amount of chlorinated congeners.

The aim of the present study was to perform a short characterization of dioxin contamination in samples from Romania of poultry liver and adipose tissue and to tentatively evaluate the ingestion of dioxins from the analyzed samples.

Materials and methods

Two different sets of poultry samples were analyzed. The first set of samples was composed of five collective commercial poultry liver samples, 3 from Romania and 2 from Belgium. For each collective samples, several livers were bought from supermarkets (3 different supermarkets in Bucharest, Romania, and2 different supermarkets, in Liège, Belgium) and were pooled together before lyophilisation.

The second set of samples was composed of liver and adipose tissues (abdominal fat) from 25 individual broilers that had macroscopic changes in the liver(hepatostatosis and multifocal miliary necrotic hepatitis). The samples from these 25 animals were taken in oneslaughterhouse in Romania, but thebroilers came from different regions of the country. The animals came from industrial intensive indoor rearing and were slaughtered at about 40 days.

Sample extraction and DR-CALUX analysis was performed as already described[2]. Briefly, all liver samples were lyophilized and the fat was extracted with hexane, using a solvent extraction. Humidity and fat content was recorded for each liver sample.

The following steps of the sample preparation were common for the fat extracted from liver and for adipose tissue. The clean-up of 2 g fat wasmade on an acidic silica column and dioxins were eluted with hexane. Residues were dissolved in DMSO.

DR-CALUX analysis was performed by exposing the cells (rat H4IIE cells from the Biodetection System company, NL) (in 96 wells plates) to samples extracts, resuspended in DMSO, during 24h before cell

lysis, and luminescence determination. A standard calibration curve was drawn for each group of samples by using standard TCDD solutions.

Results were expressed as BEQ (bioanalytical equivalents), as suggested by Hoogenboom& co-workers[6].A bovine fat sample contaminated with about 2 pg BEQ/g after spiking with the 29 congeners of dioxins, furans and dioxin-like PCBs, was used as a quality control sample.

According to the European legislation[7], the results for poultry liver were expressed per g of fat.

The limit of detection (LOD) was 0.5 pg BEQ/g fat and the limit of quantification (LOQ) was 0.9 pg BEQ/g fat.

Results and discussion

The 5 commercial liver samples showed similar humidity (about 75%) and fat content (about 4 %)

Figure 1 shows the results of the analysis of the first set of samples, the commercial samples, reported on the TCDD calibration curve.

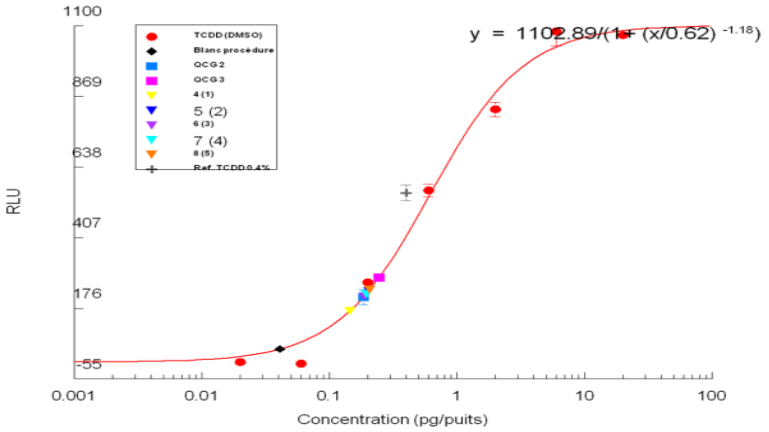

Fig. 1 Calibration curve and the results for commercial samples obtained with DR-CALUX assay

The results are presented as mean values of a triplicate analysis of the sample extract and statistical analyses were performed with SPSS software version 19 for Windows.

Also, the results were compared with the maximum levels (ML), according to Commission Regulation (EC) N° 1259/2011[7]. The ML for poultry liver are 4.5 pg $TEQg^{-1}$liver fat, for the sum of the 17 congeners of dioxins and furans, and10 pg $TEQg^{-1}$liver fat for thesum of the 29 congeners of dioxins, furans and dioxin-like PCBs.

For the 5 commercial liver samples, the results were 2.15; 2.93; 2.77; 3.14; 2. 96pg$BEQg^{-1}$ fat and all were under bothML(fig.2). Samples 1,2 and 3 were coming from Romania, while 4 and 5 from Belgium, respectively.

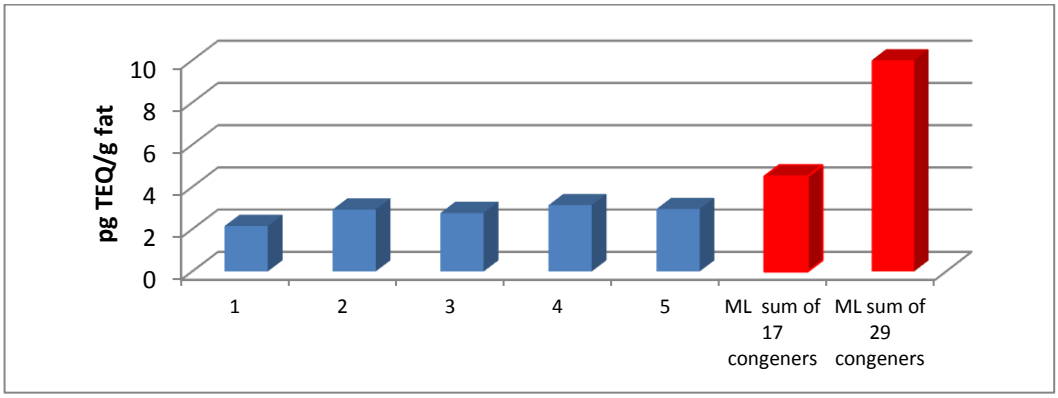

Fig.2 Results for the commercial liver samples

According to these results, we made an estimationof the daily intake of dioxins by eating 100 g fresh weight liver (a realistic consumption in Romania) and we compared with maximum accepted daily intake for a human of 60 kg, which is 120 pg TEQ/day. Concentration of dioxin in 100 g fresh sample for sample 1,2,3,4 and 5 were 3.62, 6.58, 3.99, 5.73 and 7.23 pgBEQ/ 100g sample, respectively. All these levels were well under the tolerable daily intake (TDI), representing only less than 5% of this tolerable intake.

Surprisingly, the results for samples taken in slaughterhouse (livers with macroscopic lesions and adipose tissue) were almost all under the LOQ (Table 1), and consequently all under the maximal levels according the European Legislation[7],although they came from distinct regions of Romania.

We did not measure dioxins in feed of these poultry but according to the literature, measurable quantities of these contaminants were present in commercial poultry feeds, the concentrations were generally very low[8]. Other studies[9, 10] confirmed that a source of liver contamination with dioxin is the feed.

The contribution to contaminant uptake from herbage, bedding and drinking water is considered to be important for reared indoors birds, whilesoil was considered to be the dominant sources of dioxin contamination in chickens reared outdoors, as these birds need a certain amount of dietary soil or grit to aid digestion[11].

We can not explain the difference between samples taken in supermarkets and samples taken from a slaughterhouse. On one hand, we could assume that the samples that were under the LOQ (liver with macroscopic lesions and adipose tissue) came from an unpolluted area. On an other hand, we have to consider that poultry taken in a slaughterhouse displayed liver pathologies, and we can not exclude that there is a link between these liver pathologies and the lack of dioxin bioaccumulation. This should be further investigated.

Although it is known that people ingest dioxins because of background levels especially in animal products, we show here, from a very limited study, that this intake from liver is most probably not exceeding the current TDI and thus it can be considered that there is no risk for human health linked to the consumption of poultry liver.

However, in case of accidents, when large amounts of these chemical substances are spread in the environment, dioxinscontamination represent a real risk for the consumer health, because of their bioaccumulation thought the food chainand a risk assessment should be performed, in order to quantify the exposure of humans to that precise contamination.

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Table 1 : Results for poultry samples taken in slaughterhouse in Romania.

Sample Identification	% humidity in liver	% fat in liver	pg BEQ /g fat (adipose tissue)	pg BEQ/ g fat (liver tissue)
1.1	75.50	5.61	< LOD	< LOD
1.2	77.85	3.05	< LOD	< LOQ
1.3	Not determined	Not determined	< LOD	Not determined
1.4	Not determined	Not determined	< LOD	Not determined
1.5	Not determined	Not determined	< LOD	Not determined
2.1	Not determined	Not determined	< LOD	Not determined
2.2	Not determined	Not determined	< LOD	Not determined
2.3	77.91	3.13	< LOD	< LOQ
2.4	76.65	3.87	< LOD	< LOD
2.5	Not determined	Not determined	< LOD	Not determined
3.1	Not determined	Not determined	< LOD	Not determined
3.2	Not determined	Not determined	< LOD	Not determined
3.3	77.43	2.87	< LOD	< LOQ
3.4	75.71	3.18	< LOD	< LOD
3.5	Not determined	Not determined	< LOD	Not determined
4.1	78.34	2.85	< LOD	< LOD
4.2	Not determined	Not determined	< LOD	Not determined
4.3	Not determined	Not determined	< LOD	Not determined
4.4	Not determined	Not determined	< LOD	Not determined
4.5	74.75	5.02	< LOQ	< LOD
5.1	Not determined	Not determined	< LOQ	Not determined
5.2	Not determined	Not determined	0.92	Not determined
5.3	76.46	3.10	< LOQ	< LOD
5.4	76.43	3.25	< LOQ	< LOD
5.5	Not determined	Not determined	1.09	Not determined

< LOD = < 0.5 pg BEQ / g fat

< LOQ = < 0.9 pg BEQ / g fat

ML in poultry liver [7]: 4.5 pg $TEQg^{-1}$ liver fat (WHO-PCDD/Fs-TEQ) and10 pg $TEQg^{-1}$liver fat (WHO-PCDD/FS-DL-PCBs TEQ)

ML in poultry fat [7]: 1.75 pg $TEQg^{-1}$ liver fat (WHO-PCDD/Fs-TEQ) and3 pg $TEQg^{-1}$ liver fat (WHO-PCDD/FS-DL-PCBs TEQ)

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