IMPACT OF THE NEW EU-MAXIMUM LEVELS FOR DIOXIN AND PCB ON THE ASSESSMENT OF SHEEP LIVER

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Introduction

In the years 2008-2010 a number of sheep samples were analysed in Europe for polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), in the following together termed "dioxins", and polychlorinated biphenyls (PCBs). While almost all sheep meat samples were below the then effective EU maximum levels, more than 80% of the sheep liver samples were not compliant. On request of the EU Commission, in 2011 the European Food Safety Authority (EFSA) delivered a scientific opinion on the risk to public health related to the presence of high levels of dioxins and dioxin-like PCBs (dl-PCBs) in sheep liver. EFSA concluded that the "regular consumption of sheep liver would result on average in an approximate 20 % increase of the median background exposure to dioxins and dl-PCBs for adults. On individual occasions, consumption of sheep liver could result in high intakes exceeding the tolerable weekly intake (TWI)". EFSA also concluded "that the frequent consumption of sheep liver, particularly by women of child-bearing age and children, may be a potential health concern".[1] It was also explored whether there is a need to change the basis for expression of occurrence results and maximum levels for liver from fat weight to fresh weight basis. In this respect, EFSA concluded that even if there would be a possible hepatic sequestration and the dioxins and PCBs would not be totally associated with the fat fraction of the liver, this would have no influence on the result, whether expressed on fat or fresh weight basis, as all dioxins and PCBs are extracted during the analytical procedure irrespective of the liver compartment where they are present.[1] Subsequently, the European Union Reference Laboratory (EURL) for Dioxins and PCBs in Feed and Food in Freiburg was requested by the EU Commission to investigate how different extraction methods influence the levels of dioxins and PCBs in sheep liver with regard to reporting the analytical result on fat or wet weight basis. The EURL concluded that the variations for concentrations of dioxins and PCBs were considerably higher on fat basis compared to fresh weight basis. The concentrations of dioxins and PCBs on fat basis in sheep liver were dependant on the applied extraction method or solvents and therefore on the resulting fat content. When comparing results on fresh weight basis, the levels of dioxins and PCBs were quite comparable.[2] Consequently, the EU Commission considered it appropriate to establish the new maximum levels, which are effective in the EU Memeber States since January 1, 2014, on a fresh weight basis. De facto, the new Regulation (EU) No 1067/2013[2] represents a substantial raise of the maximum levels. In order to check the impact of the new Regulation on the assessment of sheep liver, 45 liver samples were analysed for dioxins and PCBs in our laboratory. The results indicate that the levels in the current samples are comparable to those of the former analysed sheep livers. However, most of the sampes which would have exceeded the former maximum levels for dioxins and PCBs in sheep liver are now compliant with the new Regulation.

Materials and methods

The liver samples were collected in two abattoirs in North Rhine-Westphalia/Germany directly at slaughter by veterinarians. All samples were taken from lambs. Upon arrival in the CVUA-MEL, the liver samples were homogenized using a blender and an aliquot of 20 g was grinded with glass powder and sodium sulfate in a mortar. The free-flowing powder was placed in a glass column and the fat was extracted with a mixture of cyclohexane/dichloromethane 1+1 (v/v). After evaporation of the solvent, 17 $^{13}C_{12}$-labeled dioxins, 11 $^{13}C_{12}$-labeled dioxin-like PCBs (dl-PCBs) and 6 $^{13}C_{12}$-labeled non-dioxin-like PCBs (ndl-PCBs) were spiked into the fat extract. The following clean-up of the samples was performed fully automatically on an LCTech dioxin sample preparation system (DEXTech^{TM}) within 90 minutes. The clean-up system included a silica gel column coated with sulfuric acid to destroy the fat matrix, a Florisil column to separate dioxins from PCBs and 2 carbon columns to partition planar from non-planar compounds and to isolate the non-ortho PCBs from mono-ortho and di-ortho PCBs. A further $^{13}C_{12}$-labelled internal standard was spiked into each of the 3 resulting fractions

(dioxins, non-ortho PCBs, mono-ortho- and di-ortho-PCBs) and after evaporation and reconstitution of the extracts in toluene, the analytical analysis was performed by capillary gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) on a Waters AutoSpec and a Thermo-Fisher DFS system at a resolution of R=10,000. While for the dioxin analysis a DB-Dioxin column was used, the gas chromatographic separation of the PCB congeners were performed on an HT-8 capillary column. The identification of the compounds was conducted by their retention time in combination with the relative intensities of the two preselected characteristic mass fragments for each congener provided that the requirements laid down in Commission Regulation (EU) No 252/2012[3] were fulfilled. The quantification of the analytes was based on the internal standards and a five point calibration curve. Laboratory blank samples as well as quality control samples are analysed simultaneously on a regular basis. The applied analytical methods were successfully tested in a number of national and international proficiency tests.

Results and discussion

The results of the investigation are illustrated in Table 1. All levels for dioxins and dl-PCBs are calculated as toxic equivalents (WHO_{2005}-TEQ) with the human and mammalian Toxic Equivalency Factors (TEF) proposed by WHO in 2005.[4] The ndl-PCB levels represent the sum of the 6 so-called "indicator" PCB congeners 28+52+101+138+153+180 as laid down in the respective European Regulations for ndl-PCBs in food. According to the effective European legislation, the concentrations are given as upperbound values, i.e. for congeners below the limit of quantification (LOQ), the numerical value of the respective LOQ is used for the calculation of the TEQ and the ndl-PCB values. For comparison, the concentrations are given on a fresh weight basis and on a fat basis. This allows a comparison of the data related to the former maximum levels (ML) which were based on fat weight basis with the current effective ML which since the beginning of 2014 are based on fresh weight.

Parameter	**New Regulation (from 01.01.2014)**			Fat	**Old Regulation (until 31.12.2013)**		
	PCDD/F	Σ PCDD/F+dl-PCB	ndl-PCB		PCDD/F	Σ PCDD/F+dl-PCB	ndl-PCB
	pg WHO-TEQ/g fresh weight		ng/g fresh weight	%	pg WHO-TEQ/g fat		ng/g fat
Number	45			45	45		
Mean	1.37	1.89	1.8	5.0	28.4	38.9	36.5
Median	1.44	2.08	1.6	4.9	27.0	38.3	34.3
Minimum	0.15	0.21	0.1	3.5	1.8	3.4	2.3
Maximum	3.55	5.01	6.3	8.4	65.6	82.8	94.3
90^{th} perc.	2.29	2.91	2.9	6.2	50.6	66.1	61.3
ML	**1.25**	**2.0**	**3.0**	**--**	**4.5**	**10.0**	**40.0**

Table 1: Dioxins, sum of dioxins+dl PCBs, and ndl-PCBs in sheep liver. Levels are given on a fresh weight basis and on fat weight basis and evaluated with the former and new EU maximum levels (ML) according to EU Regulation (EC) No 1881/2006[5], amended by Regulation (EC) No 1259/2011[6] and Regulation (EU) No 1067/2013[2].

On average, around 70% of the total TEQs are attributed to dioxins and around 30% are attributed to dl-PCBs. In general, the concentrations of dioxins, sum of dioxins+dl-PCB, and ndl-PCB are in the same order as those which were the basis for the EFSA evaluation in 2011. The reasons for the high dioxin levels in sheep liver which in general are substantially higher than in other ruminants, such as bovine are still not clear. Based on studies in vitro and in vivo with prototype substrates for CYP1A enzymes which indicated a lower CYP1A1 activity in sheep than in cattle, EFSA concluded that differences in metabolism might be possible explanations for the marked differences in the liver storage of dioxins and related compounds between the two species. Furthermore, as demonstrated in rodents, it cannot be excluded that other mechanisms such as the sequestration of dioxins and dioxin-like compounds by hepatic CYP1A2 or their biotransformation by other enzymes, may affect their accumulation in the liver of ruminants.[1]

Taking the new Regulation which is effective from 01.01.202014 as a basis, it can be seen that the mean and median concentrations for dioxins in the present samples already exceed the respective ML. The same holds true for the median of the sum of dioxins+dl-PCB. In contrast, even the 90th percentile of the ndl-PCB concentrations is below the new ML. In summary, 15 of the 45 liver samples (33%) presently analysed exceed the new ML and 30 of the 45 liver samples (67%) are compliant with the new ML. However, 12 of the latter samples were only compliant when taking the measurement uncertainty of 20% into account. Taking the former Regulation as a basis, 39 of the 45 samples (87%) would exceed at least one of the three MLs and thus would be non-compliant. The remaining 6 samples (13%) are below the MLs and therefore would be marketable.

In conclusion, it can be stated that the dioxin and PCB levels in the present samples do not show a decrease compared to earlier liver samples analysed, but lie in the same concentration range. However, because of the new legislation with the maximum levels based on fresh weight rather than on fat weight, the number of liver samples that are below the legal limits and thus are marketable has substantially increased. In this respect, it has to be mentioned that the maximum levels for dioxins and PCBs are not toxicologically derived but are set on the frequency distribution of the contaminants in the respective matrix. This follows the principle "strict but feasible". In general, the MLs are set in the range of the 90th to 99th percentiles of the available results obtained from the respective samples collected and analysed in the European Member States.

With the new Regulation (EU) No 1067/2013, the MLs for dioxins, sum of dioxins+dl-PCB, and ndl-PCB were changed from 4.5 pg TEQ/g fat, 10 pg TEQ/g fat and 40 ng/g fat to 1.25 pg TEQ/g fresh weight, 2.0 pg TEQ/g fresh weight and 3.0 ng/g fresh weight, respectively. Taking a mean fat content of 5% into account, the new MLs would relate to 25 pg TEQ/g fat for dioxins, 40 pg TEQ/g fat for the sum of dioxins and dl-PCB and 60 ng/g fat for ndl-PCB. These values are up to 5-times higher than the former legal limits and illustrate that the new legislation does not only change the basis for reporting and evaluation of the results, but de facto also represents a substantial raise of the MLs.

The enforcement of the new MLs causes some severe problems for health authorities and surveillance bodies. Based on the EFSA risk assessment on sheep liver which concluded that the frequent consumption of sheep liver, particularly by women of child-bearing age and children, may be a potential health concern, and the fact that more than 80% of the liver samples analysed exceeded the then effective MLs, a number of enforcement bodies enacted that the sheep liver has to be discarded at slaughter unless the owner of the animal could show that the liver is compliant with the legal limits. The latter was generally not done as the costs for the analysis exceeds the value of the liver. The current investigation shows that the potential health implication has not changed as the contaminant levels in sheep liver have not appreciably changed. However around 70% of the present sheep liver are nowadays compliant compared to only around 13% when taking the former legislation as a basis. As a consequence, from a purely legislative point of view, there is no reason for a further decree to discard the sheep liver at slaughter. This discrepancy between potential health implication and compliance with legal limit is difficult to communicate to the consumer.

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6. Commission Regulation (EU) No 1259/2011, OJL 320, 02.12.2011, p. 18-23