DIOXINS AND PCBS IN PAIRED SAMPLES OF RUMINANT MEAT AND LIVER: A REVIEW OF REGULATORY CHANGES

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Introduction

In 2005 the UK Food Standards Agency carried out a survey for dioxins and PCBs in offal to assess compliance with the European regulatory limit of 6 pg WHO-TEQ/g fat that had come into force in 2002. There was an associated action level of 4 pg/g, an exceedance of which was intended to prompt a source investigation. In the case of sheep liver, 9/22 samples were non-compliant, with dioxin levels of up to 25 pg WHO-TEQ/g fat, and a further two exceeded the Action Level. There were no non-compliant bovine liver samples but 2/12 exceeded the Action Level. In ten venison liver tested, dioxin levels were in the range 13-125 pg/g fat (venison liver was not covered by the regulatory limit). The results were presented at the Dioxin 2008 symposium[1]. A proposal was made to the European Commission for a review of the limit including expression on a whole weight basis. The Commission called for further data in order to enable a review by the European Food Safety Authority (EFSA). This paper outlines the progression to revised limits for dioxins, together with new limits for total TEQ and non dioxin-like PCBs in liver, and presents new data for bovines.

Materials and methods

Paired samples of meat and liver were collected at slaughter by the Official Veterinarian Surgeon on duty at relevant slaughterhouses. For sheep (24 animals), the samples were collected at a single meat plant in Wales between September 2009 and April 2010.
All samples were supplied to the UK Food and Environment Research Agency with details of the age, gender and origin of the animal. They were analysed by high resolution gas chromatography coupled with high or low resolution mass spectrometry, for the 17 dioxin and furan congeners and 12 dioxin-like PCBs assigned Toxic Equivalency Factors by the World Health Organisation. The full methodology has been described previously.[2]

Results and discussion

The results for the paired sheep samples were originally presented at Dioxin 2012[3] and are reproduced in Table 1 to allow a comparison with the more recent bovine data, which are provided below, in Table 3. The data in Table 1 are ordered on the basis of the total TEQ levels in the muscle fat. All of the shoulder meat samples were compliant with the existing European regulatory limits for dioxins and dioxins plus dioxin-like PCBs, of 2.5 and 4.0 pg WHO_{2005}-TEQ/g fat[4] and were not even approaching the corresponding Action Levels of 1.75 pg/g for both dioxins and dioxin-like PCBs. This indicates that none of the animals had been exposed to atypical levels of contamination. In contrast, 10 out of 24 sheep liver samples (shaded boxes in Table 1) were non-compliant with the limits for dioxins and dioxins plus dioxin-like PCBs in liver of 4.5 and 10.0 pg WHO_{2005}-TEQ/g fat, respectively, which were introduced in 2011 and were in force at the time the results were obtained.[4] All revisions to limits at the time were associated almost entirely with the transition from the 1998 WHO-TEFs to the 2005 values rather than on the basis of fresh data.

In July 2011, EFSA published its opinion based on a substantial new dataset for sheep liver. Although they used an average liver TEQ concentration of 26.1 pg/g fat, which is considerably higher than the mean value of 7.8 pg/g fat found by the UK (Table 1), EFSA concluded that only frequent consumption of sheep liver by women of child-bearing age and children might be a health concern[5]. However, frequent consumption of liver is already contrary to official advice due to concerns about excessive vitamin A exposure. EFSA had also been asked to comment on whether it would be appropriate to express the limits in liver on a whole weight basis. Their recommendation was that, whilst this would

Table 2. Liver limits based on whole weight

	Sum of dioxins (WHO-PCDD/F-TEQ)	Sum of dioxins and dioxin-like PCBS (WHO-PCDD/F-PCB-TEQ)	Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6)
Liver of terrestrial animals referred to in 5.1 with the exception of sheep and derived products thereof	0.30 pg/g wet weight	0.50 pg/g wet weight	3.0 ng/g wet weight

be preferable for risk assessment purposes, it would be inappropriate to change the basis of the limit for liver without doing the same for other limits. Subsequently, the European Union Reference Laboratory for Dioxins investigated different extraction techniques for sheep liver. It was found that solvent systems and extraction conditions used for liver analysis have a significant influence on the results for dioxins and PCBs expressed on a fat basis, with a coefficient of variation (cv) of 24-29% for 15 different extractions of the same sample whilst the cv was reduced to 15-18% when the results were recalculated on a whole weight basis (the cv for the whole weight results reduced further when acetone-based solvent mixtures were excluded)[6]. The cv was similar to that for the different fat extraction efficiencies, suggesting that the extraction of dioxins and PCBs was less solvent-dependent than fat. Although the EURL concluded that the extraction method used for sheep liver analysis would therefore need to be very precise (and would mean many analytical laboratories probably having to change methods that had already been accredited), the Commission preferred the simpler option of moving to limits expressed on a whole weight basis. The new limits for liver were finally introduced in October 2013[7]. These are set out in Table 2.

The new sheep liver limits, supported by a substantial dataset, were placed in a separate category. There was insufficient data for other species, which were simply converted from the fat-based limit using an estimated average fat content. Concern remained about whether other ruminants also accumulated dioxins in liver in the same way as sheep. Furthermore, the lifting of the Over Thirty Month (OTM) rule that was in place in the UK, originally introduced to counter concerns about TSE,

Table 3. Results for bovine meat and liver, pg WHO-TEQ/g (upper bound)

Age	Shoulder, fat			Liver, fat			Liver, whole		
	Dioxin	PCB	Total	Dioxin	PCB	Total	Dioxin	PCB	Total
9	0.06	0.06	**0.11**	0.62	0.21	**0.83**	0.02	0.02	**0.04**
6	0.11	0.06	**0.16**	1.60	0.40	**2.00**	0.04	0.02	**0.06**
13	0.11	0.08	**0.18**	1.51	0.46	**1.97**	0.03	0.02	**0.04**
0	0.12	0.09	**0.21**	2.47	1.06	**3.53**	0.05	0.03	**0.08**
10	0.11	0.17	**0.28**	0.82	0.65	**1.47**	0.02	0.03	**0.05**
11	0.18	0.11	**0.29**	1.64	0.47	**2.11**	0.05	0.02	**0.07**
10	0.18	0.12	**0.30**	0.71	0.28	**0.99**	0.02	0.02	**0.04**
2	0.17	0.17	**0.34**	0.76	0.34	**1.10**	0.02	0.02	**0.04**
3	0.19	0.17	**0.36**	1.04	0.34	**1.38**	0.03	0.02	**0.05**
1	0.20	0.17	**0.37**	1.13	0.39	**1.52**	0.05	0.03	**0.07**
3	0.26	0.18	**0.44**	1.68	0.61	**2.29**	0.04	0.03	**0.07**
2	0.31	0.20	**0.51**	4.77	0.48	**5.25**	0.16	0.03	**0.19**
8	0.26	0.30	**0.56**	1.12	0.56	**1.68**	0.04	0.03	**0.07**
13	0.31	0.26	**0.57**	0.97	0.51	**1.48**	0.03	0.03	**0.06**
4	0.34	0.24	**0.58**	3.80	1.35	**5.15**	0.08	0.04	**0.12**
1	0.31	0.30	**0.61**	1.67	0.73	**2.40**	0.05	0.03	**0.08**
2	0.37	0.26	**0.63**	4.74	0.67	**5.41**	0.14	0.03	**0.17**
15	0.28	0.36	**0.64**	1.65	0.78	**2.43**	0.05	0.03	**0.08**
5	0.33	0.41	**0.74**	2.21	1.15	**3.36**	0.07	0.05	**0.12**
7	0.47	0.30	**0.77**	5.17	1.24	**6.41**	0.16	0.05	**0.21**
4	0.39	0.44	**0.83**	2.00	1.20	**3.20**	0.04	0.04	**0.08**
9	0.59	0.42	**1.01**	3.17	1.44	**4.61**	0.08	0.05	**0.12**
2	0.82	0.72	**1.54**	4.17	1.81	**5.98**	0.11	0.05	**0.16**
7	0.75	0.95	**1.70**	4.28	2.37	**6.65**	0.13	0.07	**0.21**
12	1.02	0.95	**1.97**	8.06	3.30	**11.36**	0.20	0.09	**0.29**
Min	0.06	0.06	**0.11**	0.62	0.21	**0.83**	0.02	0.02	**0.04**
Mean	0.33	0.30	**0.63**	2.47	0.91	**3.38**	0.07	0.03	**0.10**
Max	1.02	0.95	**1.97**	8.06	3.30	**11.36**	0.20	0.09	**0.29**

meant that bovine meat and liver could be entering the food chain from animals of an age for which no data had previously been generated and therefore no account could have been taken of any age-related bioaccumulation of dioxins and PCBs. Consequently, a similar investigation to that for sheep was carried out for bovines, using paired samples of meat and liver (25) collected at various plants around the UK between December 2013 and March 2014. The analytical results are set out in Table 3.

As in the case of sheep, no meat samples exceeded the regulatory limits for dioxins and dioxin-like PCBs, and none were approaching the Action Levels, indicating that none of the animals had been exposed to atypical levels of contamination. For the liver samples, four of 25 (shaded cells) were above the previous limit of 4.5 pg WHO-TEQ/g fat although only one would have slightly exceeded the limit once measurement uncertainty was taken into account. Also in the liver, the highest level for the sum of

Table 1. Results for sheep meat and liver, pg WHO-TEQ/g (upper bound)

Age	Shoulder, fat			Liver, fat			Liver, whole		
	Dioxin	PCB	Total	Dioxin	PCB	Total	Dioxin	PCB	Total
4	0.10	0.08	**0.19**	3.62	1.77	**5.38**	0.18	0.09	**0.27**
1	0.12	0.07	**0.19**	4.31	1.07	**5.38**	0.28	0.07	**0.35**
1	0.09	0.11	**0.21**	0.65	0.47	**1.12**	0.06	0.07	**0.13**
6	0.09	0.12	**0.21**	1.62	1.04	**2.65**	0.09	0.05	**0.13**
5	0.09	0.15	**0.25**	2.01	1.49	**3.50**	0.10	0.06	**0.16**
2	0.17	0.08	**0.25**	3.13	1.13	**4.26**	0.20	0.07	**0.27**
5	0.13	0.14	**0.28**	1.74	1.04	**2.77**	0.10	0.06	**0.15**
5	0.18	0.16	**0.34**	3.05	1.77	**4.81**	0.19	0.11	**0.30**
5	0.21	0.14	**0.35**	3.04	1.39	**4.43**	0.27	0.12	**0.39**
1	0.20	0.16	**0.36**	1.80	1.28	**3.09**	0.10	0.07	**0.17**
1	0.20	0.22	**0.42**	**4.87**	3.42	**8.29**	0.26	0.18	**0.43**
2	0.26	0.18	**0.45**	**5.79**	1.86	**7.65**	0.24	0.07	**0.31**
3.5	0.23	0.23	**0.46**	2.32	1.79	**4.11**	0.24	0.19	**0.43**
4	0.28	0.18	**0.46**	2.03	0.74	**2.77**	0.18	0.07	**0.25**
6	0.28	0.18	**0.47**	**5.07**	1.72	**6.79**	0.27	0.09	**0.36**
4	0.27	0.29	**0.55**	**5.06**	2.81	**7.86**	0.25	0.14	**0.39**
1	0.36	0.25	**0.61**	2.08	0.89	**2.97**	0.14	0.06	**0.21**
0.33	0.41	0.23	**0.64**	3.41	0.89	**4.30**	0.25	0.07	**0.31**
5	0.47	0.30	**0.77**	**5.24**	2.48	**7.72**	0.32	0.15	**0.46**
0.33	0.53	0.25	**0.78**	**6.14**	1.50	**7.64**	0.32	0.08	**0.40**
2	0.57	0.34	**0.91**	**9.35**	2.84	**12.2**	0.63	0.19	**0.81**
0.33	0.68	0.42	**1.10**	**6.04**	2.45	**8.48**	0.27	0.11	**0.38**
6	0.82	0.37	**1.19**	**28.9**	7.66	**36.5**	1.40	0.37	**1.77**
0.25	0.94	0.49	**1.43**	**15.2**	4.61	**19.9**	0.83	0.25	**1.08**
Min	0.09	0.07	**0.19**	0.65	0.47	**1.12**	0.06	0.05	**0.13**
Mean	0.33	0.21	**0.54**	5.75	2.09	**7.84**	0.32	0.11	**0.43**
Max	0.94	0.49	**1.43**	28.9	7.66	**36.5**	1.40	0.37	**1.77**

the ICES6 marker PCBs was 0.4 ng/g whole weight compared with a limit of 3.0 ng/g, and they are not further discussed here.

For sheep, there were two indications that dioxins and PCBs accumulate differently in shoulder fat and liver. Firstly, the dioxin/PCB ratios were different for the two tissue types, the ratios being consistently higher in liver, suggesting preferential binding of dioxin/furan congeners. This was also the case for bovines. The dioxin/PCB ratios are shown in Table 4.

Secondly, in sheep there were notable differences in the congener profiles between shoulder fat and liver, with predominance of furans in the latter. In bovines, the predominance of furan congeners was less marked.

Table 4.

	Shoulder ratio		Liver ratio		Liver ratio / shoulder ratio	
	Bovine	Sheep	Bovine	Sheep	Bovine	Sheep
Min	0.65	0.62	1.26	1.30	0.18	1.09
Mean	1.23	1.40	3.02	2.44	0.84	1.75
Max	2.20	2.25	9.94	4.09	3.07	2.33

For sheep, there appeared to be little major influence of age on the levels of dioxin and PCB accumulation in meat or liver and this was also the case for bovines, which is important in the context of controls over human dietary exposure. However, to verify that there is no age-related build-up, it

would be necessary to monitor individual animals over an extended period, which is outside the context of the current work.

In conclusion, as with sheep there are clear differences between the accumulation of dioxins and PCBs in liver and other tissue in bovines, which may be accounted for by protein-binding within the liver. However, while levels in meat fat remain similar for sheep and bovines, average levels in sheep liver are higher, justifying the establishment of higher regulatory limits for the latter. In neither case is there an age-related effect, which is important in the context of allowing meat from bovines over thirty months to enter the food chain.

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References:
1. Mortimer DN, Gem M, Rose, MD, Fernandes A, White S. (2008) *Organohalogen Compounds* 70: 883-886.
2. Fernandes A, White S, D'Silva K, Rose M. (2004) *Talanta* 63: 1147-1155.
3. Mortimer D. (2012) *Organohalogen Compounds* 74: 739-742.
4. Commission Regulation (EC) No. 1259/2011. *Official Journal of the European Union* L320: 18-23.
5. EFSA Panel on Contaminants in the Food Chain (CONTAM) (2011) EFSA Journal 9(7): 2297.
6. Kotz A, Malisch R, Wahl K, Haedrich J. (2012) Organohalogen Compounds 74: 160-163.
7. Commission Regulation (EU) No 1067/2013. *Official Journal of the European Union* L289: 56-57.