STUDY OF A RELATIONSHIP BETWEEN PCB CONCENTRATIONS IN BOVINE SERUM, FAT AND MUSCLE SAMPLES

Marchand P[1], Vénisseau A[1], Brosseau A[1], Gadé-Hildevert C[1], Ramdin F[1], Le Bizec B[1]

[1]Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA)-Ecole Nationale Vétérinaire de Nantes (ENVN) - Route de Gachet - BP 50707 - 44307 Nantes Cedex 3 – France – laberca@vet-nantes.fr

Abstract
The purpose of the present study was to analyse four different kinds of bovine matrices (liver, serum, fat and muscle samples) in order to investigate the relationship between the PCB concentrations in those different compartments of the animal. A total of 12 slaughtered castrated males were studied. In all 12 of them, we analysed the PCB level in blood as well as in a pool of muscles and in 4 of them, we made a complete analysis of muscle, fat, liver and blood samples. Our results seem to show no difference statistically significant in terms of PCB levels in all fat tissues and in 3 of the 4 muscle tissues. We demonstrated a correlation between the PCB levels of fat taken by biopsy from behind the ear of the animal and the muscles. We also showed a good correlation between the results for blood and the pool of muscles.

Introduction
Although polychlorinated biphenyls (PCB) have been banned in all industrialised countries for more than 30 years they may still be present in most of the environmental matrices in Northern countries. The consequence is that those compounds may be present at a more or less high level in animals, which could cause concern for human health. The current regulation regarding dioxins and PCB in food is based on maximal tolerable limits in various edible tissues or products, the advantage being that the Authorities have fixed a maximum limit for each kind of matrix. However, one of the drawbacks of this system is that it is difficult to perform an efficient and rapid control on live animals. Indeed, the determination of PCDD/F and PCB levels in muscle clearly implies animal slaughtering. On live animals, however, serum is recognized as a good indicator of exposure and is easy to collect. But since it is not directly consumed or used, no limits have been established for this matrix. Moreover, maximum limits have been fixed in fat and muscles, but no correlation has been demonstrated between all the classes of these matrices. In this context a few questions need to be asked:

- Is there homogeneity of the tissues?
- Is the result of a biopsy predictive of the value we could find in the muscle?
- Is it possible to consider blood as representative of the charge?

Materials and Methods
Samples
Animals were castrated males between 3 and 4 years old naturally grazed with hay contaminated by PCB. Twelve animals were slaughtered and 10 samples were taken on each animal in order to compare the contamination. Different kinds of muscles were taken from the neck, the shoulder, the prime cut of the beef (thick skirt or hanging tender) and finally on the topside (outside flat or bottom round). For the fat, internal fat was taken (peritoneal and perirenal) and external fat corresponding to superficial fat was taken from an easy collection spot (fat from the ear and the neck). A sample of blood and a part of the liver were also collected. The 12 blood samples and 12 muscle pools were analyzed, but only 4 animals were completely studied.
Extraction and clean-up
Blood sample was collected without an anticoagulant, centrifugated and the upper layer corresponding to the serum was withdrawn. The 18 ^{13}C-labelled standards (12 dioxin-like PCB and 7 markers PCB) were added to the serum sample before extraction. After spiking, the sample was diluted with deionised water. Extraction procedure was performed as follows: addition of aqueous saturated ammonium sulphate and ethanol, extraction twice with hexane. The total lipid content of the serum samples was determined using an enzymatic dosage of four classes of lipids on a 50µL aliquot. Before extraction, muscle and liver samples were freeze-dried and the internal standards were added. The extraction was performed using the Accelerated Solvent Extractor (ASE) with a toluene/acetone mixture (70/30, v/v). The solvent was evaporated to dryness, allowing an estimation of

the fat content. Fat samples were put in an oven at 105°C overnight and fat was directly taken using a Pasteur pipette. Clean-up and separation processes were carried out using the classic liquid-solid adsorption chromatography with silica, Florisil and CarbopackC/Celite. The solvent used for the elution was hexane. The external standard was added for the recovery calculation ($^{13}C_{12}$-PCB #111 for the 2 fractions of PCB- planar and non planar PCB).

GC/HRMS analysis

GC/HRMS analysis of the 12 dioxin-like and 7 markers PCB was performed as previously described[1]. The congeners were separated by gas chromatography (GC) on a DB-5MS capillary column (30 m × 0.25 mm, 0.25 µm) and determined by high-resolution mass spectrometry (HRMS) on a JMS 700D (Jeol), at a resolution of 10000 in the selected ion-monitoring (SIM) mode using Electronic Impact as ionisation technique. TEQ values were calculated using WHO-TEFs.

Results and Discussion

The PCB concentrations measured in all the samples are presented in Table 1.

animal	matrix	DL-PCB WHO-TEQ (pg/g of fat)	Sum marker PCB (ng/g of fat)	fat content (%)
1900	perirenal fat	2,95 ± 0,60	15.99 ± 3.63	95.09
	peritoneal fat	2,94 ± 0,60	17.29 ± 3.92	94.76
	neck fat	2,96 ± 0,60	15.56 ± 3.53	81.93
	ear fat	2,65 ± 0,54	14.10 ± 3.20	77.96
	neck meat	2,53 ± 0,52	14.46 ± 3.28	6.74
	topside meat	2,47 ± 0,50	14.21 ± 3.22	2.89
	shoulder meat	2,39 ± 0,49	14.00 ± 3.18	6.76
	prime cut meat	2,46 ± 0,50	14.41 ± 3.27	10.30
	pool of muscles	*2,39 ± 0,49*	*13.81 ± 3.13*	*7.61*
	liver	2,91 ± 0,59	39.01 ± 8.85	5.74
1912	perirenal fat	71,32 ± 14,56	193.01 ± 43.79	87.58
	peritoneal fat	70,67 ± 14,43	190.30 ± 43.18	90.38
	neck fat	61,56 ± 12,57	162.66 ± 36.91	70.94
	ear fat	66,70 ± 13,62	176.38 ± 40.02	87.15
	neck meat	55,69 ± 11,37	181.19 ± 41.11	5.56
	topside meat	40,87 ± 08,35	138.17 ± 31.35	1.12
	shoulder meat	51,72 ± 10,56	169.20 ± 38.39	3.05
	prime cut meat	57,77 ± 11,80	187.03 ± 42.44	8.24
	pool of muscles	*49,09 ± 10,03*	*153.89 ± 34.92*	*5.34*
	liver	103,59 ± 21,15	387.50 ± 87.92	5.54
1913	perirenal fat	23,36 ± 4,77	82.38 ± 18.69	91.87
	peritoneal fat	20,31 ± 4,15	71.33 ± 16.19	92.45
	neck fat	21,31 ± 4,35	77.18 ± 17.51	77.58
	ear fat	22,03 ± 4,50	78.80 ± 17.88	88.09
	neck meat	21,48 ± 4,39	90.92 ± 20.63	5.78
	topside meat	16,14 ± 3,30	69.25 ± 15.71	2.14
	shoulder meat	20,68 ± 4,22	86.68 ± 19.67	4.18
	prime cut meat	22,66 ± 4,63	94.59 ± 21.46	5.85
	pool of muscles	*23,64 ± 4,83*	*100.15 ± 22.72*	*3.95*
	liver	36,37 ± 7,43	169.13 ± 38.37	5.61
4633	perirenal fat	15,68 ± 3,20	53.99 ± 12.25	95.66
	peritoneal fat	18,54 ± 3,79	61.55 ± 13.96	93.87
	neck fat	12,38 ± 2,53	40.64 ± 9.22	75.60
	ear fat	15,12 ± 3,09	48.49 ± 11.00	79.72
	neck meat	14,22 ± 2,90	57.97 ± 13.15	3.65
	topside meat	09,95 ± 2,03	40.81 ± 9.26	1.34
	shoulder meat	13,49 ± 2,76	53.25 ± 12.08	3.51
	prime cut meat	15,99 ± 3,27	61.94 ± 14.05	6.20
	pool of muscles	*14,89 ± 3,04*	*57.91 ± 13.14*	*4.39*
	liver	23,91 ± 4,88	85.63 ± 19.43	5.57

Table 1: DL PCB-WHO-TEQ and marker PCB sum in muscle, liver and fat samples.

The values determined are in the same range for all the samples originating from the same animal. We can observe that there is no statistically significant difference between three of the muscles following the ANOVA test. For one of them, if we consider the 3 animals whose concentrations were clearly above the maximum limit, the deviation between the topside and the prime cut meat samples was systematically higher than 28% (respectively 28.8, 29.2 and 37.8). Despite this observation, based on the data obtained, the fat content of the muscles may influence the PCB levels.

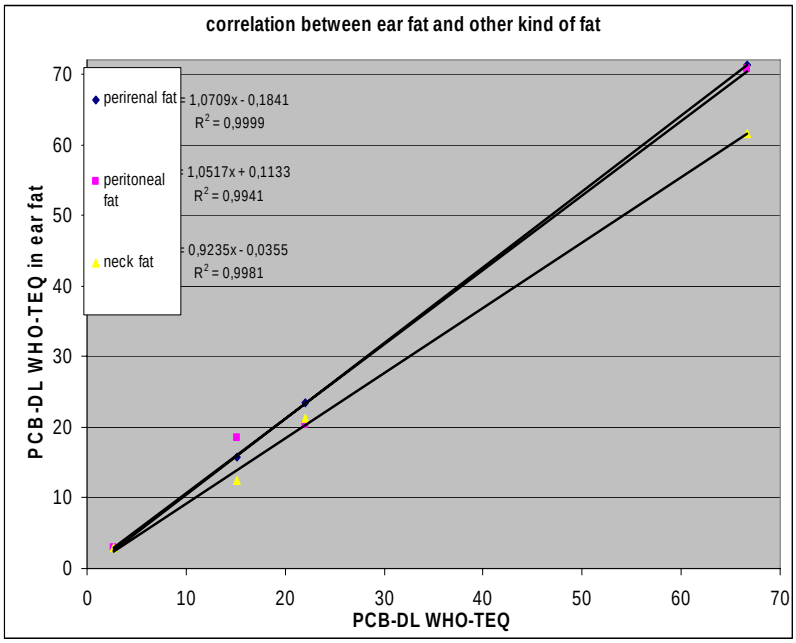


Fig 1: correlation between ear fat and the others classes of fat

Fig 1 shows the relationship between the different kinds of fat. The ear fat was taken as the reference because it is directly accessible by biopsy and the quantity of fat which can be taken at this spot is high.
The correlation found is very good with an excellent correlation factor, which means that this kind of sample could reasonably be allowed for an evaluation of the contamination degree and probably statute on the compliancy of the animal.

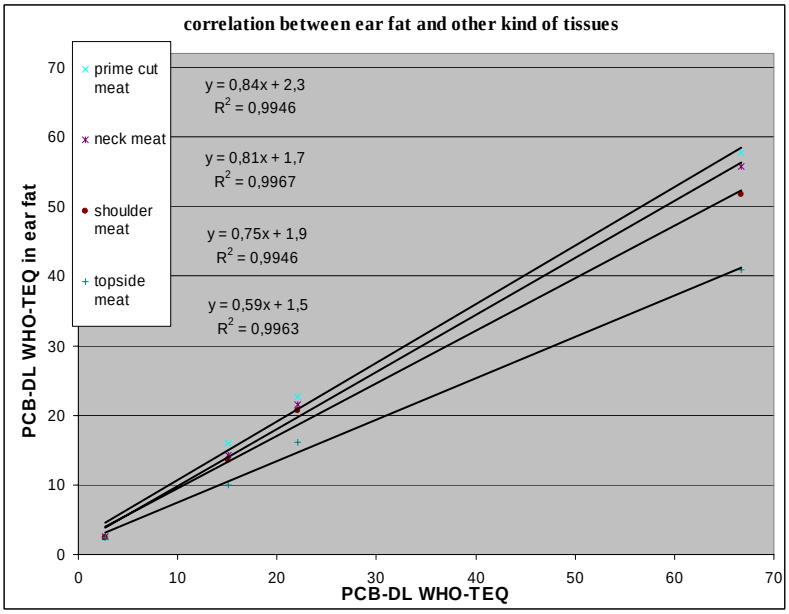


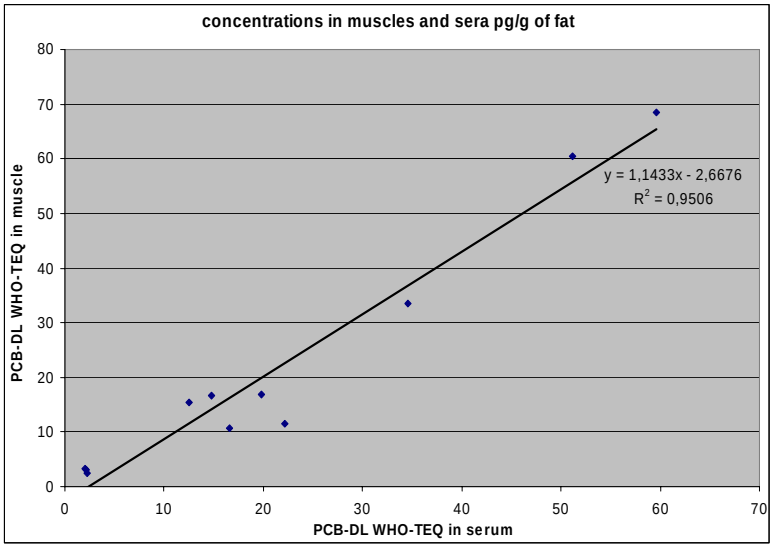
Fig 2: relationship between ear fat and the 4 different muscles

The comparison of the results between the different kinds of muscles and the ear fat revealed high correlation factors meaning that ear fat could be a good indicator of the PCB body burden (fig2). However we must not minimize the fact that only the 3 fattiest muscles are close to the value found in the ear fat in terms of concentration.
As an illustration of this comment, the deviation between the mean of the 3 fattiest muscles and the leanest one is in a magnitude of 20%.

It would be interesting to qualify the status of an animal while it is still alive. The fat from the ear taken by biopsy produces an interesting result but this can also be done by blood collection. The correlation between serum and the pool of the 4 kinds of muscles has been studied. We chose to compare serum and the pool of muscles. Results are presented in Table 2 and Fig 3.

Animal	muscle		blood	
	DL PCB (pg/g fat)	marker PCB (ng/g fat)	DL PCB (pg/g fat)	marker PCB (ng/g fat)
1900	2.09	16.84	3.14	22.94
1906	2.29	12.69	2.42	18.68
1907	1.99	14.45	3.22	26.01
1910	14.81	43.05	16.64	62.94
1912	59.57	196	68.47	252.66
1913	22.15	100.72	11.61	66.69
1914	16.58	58.35	10.63	41.79
1915	34.61	127.34	33.44	148.93
1920	51.21	225.44	60.51	321.23
4633	12.56	55.51	15.43	80.85
4635	19.76	88.1	16.82	96.22

Table 2: DL PCBWHO-TEQ and marker PCB sum in muscle and blood samples.



As expected, there is a very good correlation between the serum and the pool of muscles whatever the level of contamination.

Fig 3: Observed correlation between the PCB concentrations in serum and muscle

Conclusion

According to this study we have observed that all matrices (except for liver) originating from the same animal are in the same range in terms of concentration. In some cases, the results show no difference statistically significant in PCB levels in fat tissues and muscle tissues. To conclude, it seems relevant to consider that bovine ear fat obtained by biopsy or blood samples can be used to statute on the compliancy of the animal.

Acknowledgements

We would like to thank the DGAl for its financial support and the French Departmental Veterinary Services and the National Interprofessional Center for the Dairy Economy for their contribution to this work.