IS THE DIOXIN CONTAMINATION LEVEL IN BOVINE MUSCLE PREDICTABLE FROM LESS INVASIVE BIOLOGICAL SAMPLES?

P. Marchand, A. Vénisseau, A. Brosseaud, C. Gadé-Hildevert, JP Antignac, B. Le Bizec

Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), USC INRA 2013, Ecole Nationale Vétérinaire de Nantes (ENVN), BP 50707, 44307 Nantes Cedex 3, France (laberca@vet-nantes.fr)

Introduction

In spite of huge efforts made for several years to reduce industrial emissions of dioxins and PCB in most of developed countries, some specific situations of acute contamination may still arrive. In most of cases, the origin of such accidental events appears in relation with ingredients in feed (ball clay, guar, citrus pellet….) or due to the vicinity with plants industry releasing pollutants. The current regulation regarding dioxins and PCB in food is based on maximal tolerable limits established for various edible tissues or products. However, one drawback of this system is that such control of edible matrices is non achievable from living animals. As typical example, the determination of PCDD/F and PCB levels in muscle clearly always implies animal slaughtering.

In 2007, a particular case of contamination was discovered in the west part of France that implicated a few farms. The corn silage entering in the feed composition of the lactating cows was rapidly identified as the source of this contamination. Then, authorities naturally asked the farmer to stop feeding animals with this contaminated corn, and the contamination decrease was rapidly observed in cow's milk. Besides this observation, questions a real concern arises regarding the sanitary status of non-lactating animals which don't have the ability to excrete the pollutants through this biological route. Indeed, no solution may be proposed in this case to evaluate the residual contamination levels in meat without slaughtering the animals. In this context, the aim of the present study was to investigate the relationship between the dioxin concentrations in different compartments of the animal in order to determine in what extend it would be possible to statute on the compliancy of the animal before the slaughtering.

Materials and Methods

Samples

The experimental material used for this study included biological samples collected from a total of 14 animals. For most of them, PCDD/PCDF were determined in blood (individual samples), muscle (pool samples), and 4 sorts of fat tissue. Peripheric kidney fat was chosen as model of internal fat. The additional 3 subcutaneous fat samples were located behind the ear, near the sternum and sub-caudal, respectively. Animals were for 11 of them castrated males between 15 and 18 months old naturally grazed with the corn silage identified as contaminated by dioxins. The 3 last animals were calves fed with the milk produced in the farm by contaminated lactating cows. All animals were slaughtered and different muscle samples were collected from the neck, the prime cut of the beef (thick skirt or hanging tender) and finally on the topside (outside flat or bottom round). Internal and subcutaneous fat samples were also collected as well as blood samples.

Extraction and clean-up

Blood samples were collected without using any anticoagulant agent. Samples were centrifuged and the upper layer corresponding to the serum was collected. The 17 ^{13}C-labelled internal standards compounds used for PCDD/PCDF quantification were added to each serum sample before extraction. After spiking, samples were diluted with deionised water. Briefly, the extraction procedure used included an addition of aqueous saturated ammonium sulphate and ethanol, and a double liquid extraction with hexane. The total lipid content of each serum samples (50µL aliquot) was determined using an enzymatic dosage of four classes of lipids.

The extraction for fat samples was performed using the Accelerated Solvent Extractor (ASE) with a toluene/acetone mixture (70/30, v/v). After evaporation of the solvent to dryness, the fat residue extract was put in an oven at 105°C overnight. Clean-up and separation processes were carried out using a classically used liquid-solid adsorption chromatography with silica, Florisil and CarbopackC/Celite, successively. Hexane was used as final solvent for eluting PCDD/PCDF. $^{13}C_{12}$-1,2,3,4-TCDD was used as external standard for the recovery calculation and was added in each final extract before injection.

GC-HRMS measurement

Gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) was used for identification and quantification of the 17 PCDD/PCDF congeners, through a method previously described[1]. GC separation was performed on a DB-5MS capillary column (30 m × 0.25 mm, 0.25 µm. HRMS measurement was achieved on a JMS 700D electromagnetic instrument (Jeol, Japan), operating at a resolution of 10,000 in the selected ion-monitoring (SIM) acquisition mode after Electron Impact ionisation (EI). TEQ values were classically calculated according to WHO-TEFs recommendations.

Results and Discussion

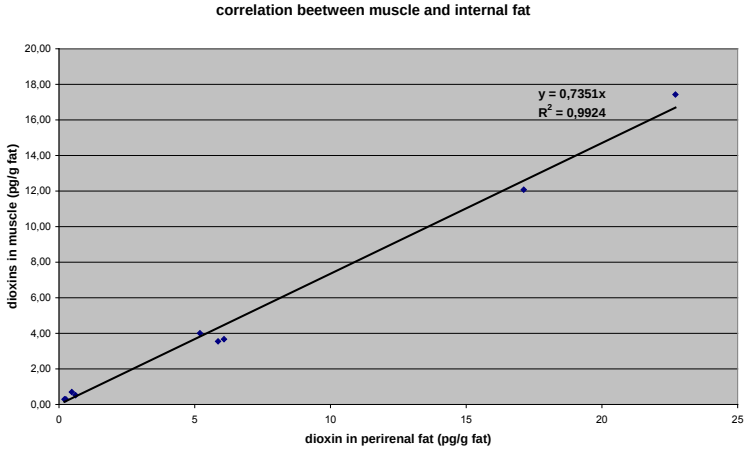
The WTO-TEQ values obtained for each analysed muscle, fat and blood samples are presented in Table 1.

Table 1: PCDD/F-WHO-TEQ values obtained for each analysed muscle, fat and blood samples.

Type of animal	Animal Identification	sub-caudal fat WHO-TEQ (pg/g fat)	Peripheric kidney fat WHO-TEQ (pg/g fat)	blood WHO-TEQ (pg/g fat)	muscle WHO-TEQ (pg/g fat)
beef cattle	5953	0.2	0.25	-	0.29
beef cattle	5957	0.25	0.2	0.98	0.29
beef cattle	2082	0.74	0.47	1.26	0.70
beef cattle	9111	0.8	0.61	1.15	0.53
beef cattle	4426	4.95	5.19	5.15	4.01
beef cattle	6372	4.97	5.86	3.75	3.55
beef cattle	9073	6.04	6.08	6.47	3.67
calf	7551	6.32	6.4	5.16	-
calf	6368	7.51	7.32	5.69	-
beef cattle	2009	8.32	9.32	7.52	-
beef cattle	2015	9.75	10.95	9.77	-
beef cattle	5304	17.82	17.12	18.85	12.08
calf	8263	20.92	22.71	21.92	17.43
beef cattle	7372	24.52	21.59	23.24	-

Correlation between muscles and internal fat

Animals included in this study came from 6 distinct farms. The concentration given for each muscle sample corresponds to the average of 3 replicates (different aliquots). From a general point of view, it was noticed that the concentration observed in muscle appears systematically from 30 to 40% below the concentration measured in internal fat (Fig. 1).

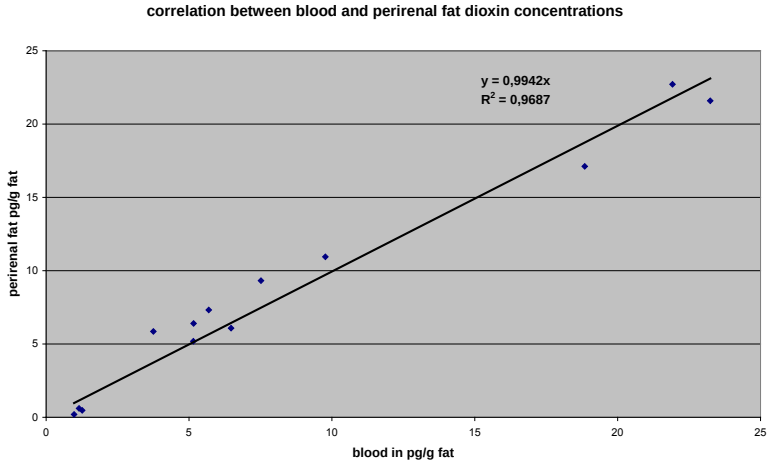


This characteristic has already been observed for dioxin-like PCBs. From a legal point of view this observation has not been taken into account as maximum limits are the same for fat and muscle. This could lead to accept the carcass but not the superficial or internal fat coming from the same animal. To avoid this bias we preferred to take a decision for the compliancy of the animal on the basis of the more contaminated compartment (peripheric kidney fat).

Fig. 1: Correlation observed between PCDD/F concentration levels measured in muscle, and peripheric kidney fat samples.

Correlation between muscles and internal fat

Results obtained for blood and internal fat samples are presented in Fig. 2.

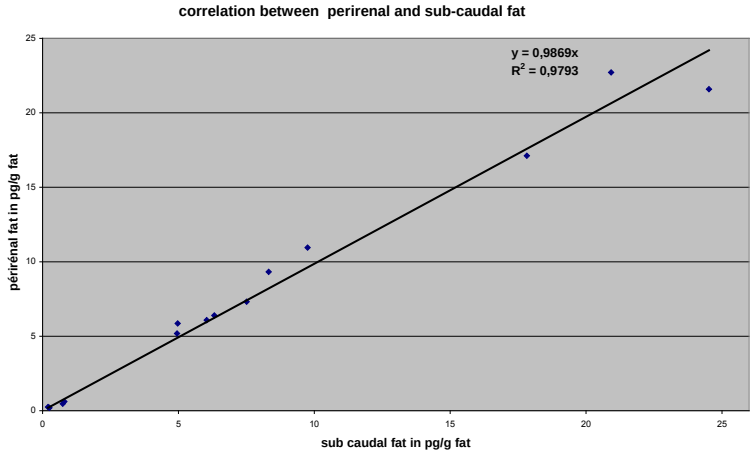


As expected, a very good correlation was found between serum and internal fat whatever the considered contamination level. The highly significant coefficient of determination R^2 indicates that this kind of sample could reasonably be allowed for an evaluation of the contamination degree and probably statute on the compliancy of the animal even if no limits has been fixed for serum.

Fig 2: correlation between PCDD/F in blood, and peripheric kidney fat samples.

Correlation between external fat and internal fat

Finally correlation between internal and external fat are exposed in Fig. 3.



Our results seem to show no difference statistically signify-cant in terms of dioxin levels in the different kind of fat. The contaminants circulation via the blood expose all the tissues and compartments at the same level. Because purification steps, and results expression in the same unit are similar no differences are observed.

Fig 3: correlation between PCDD/F in peripheric kidney fat and subcutaneous samples.

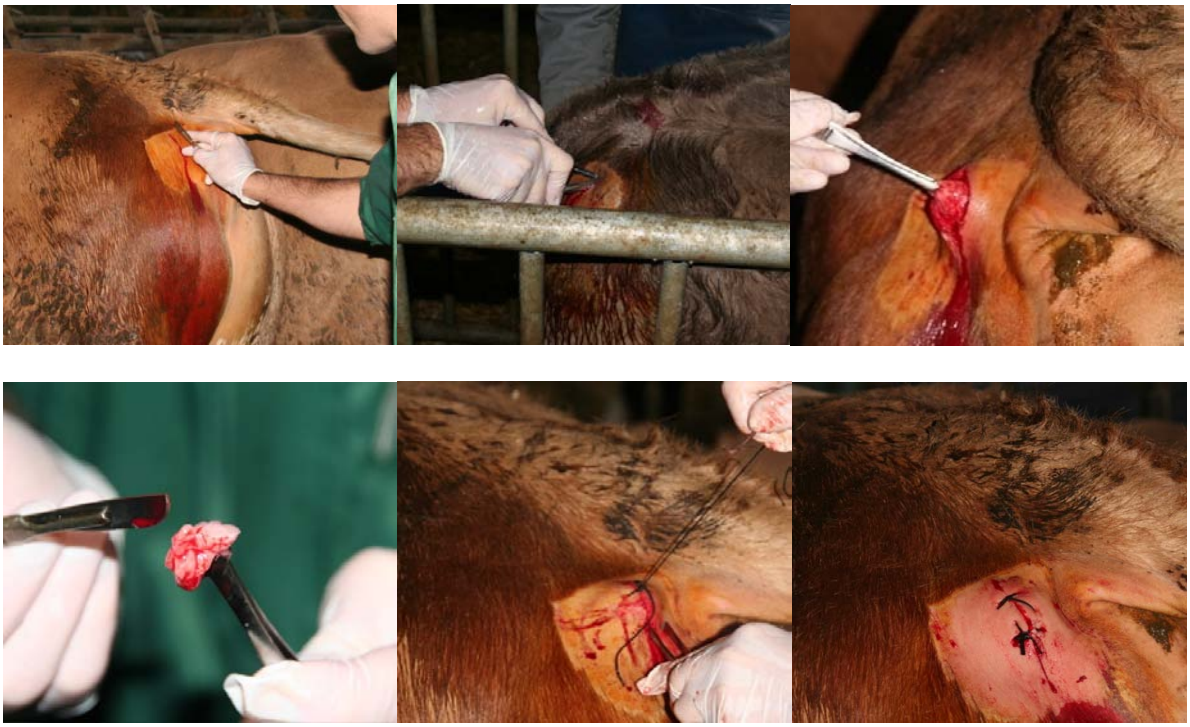
Protocol used for the crisis

The main conclusions of this study can be summarized as following:

- a very good correlation between the dioxins level of sub-caudal fat taken by biopsy and internal fat from the same animal was demonstrated,
- a good correlation between the results for blood and internal fat was also observed,
- no significant difference was observed in terms of PCDF/PCDD concentrations for 2 animals coming from the same farm and fed with the same alimentation.

According those results, a biopsy of fat as well as blood sample collection should be proposed as useful and efficient method to statute on the compliancy of the animal. Considering that fat sample is in some extend easy to collect and clearly easier to analyze than blood, biopsy may be finally retain as a method of choice. Indeed only 2 days are needed to give a result for a fat sample instead of 3 or 4 for serum sample. It was asked the

veterinarian to collect for a minimum of 0.5 g of fat. An equation was used to convert the level found in subcutaneous fat to the value we should had find if we had kidney fat. (kidney fat = 0.987 x subcutaneous fat). It was also suggested that a minimum of 10 % of animals in the same box, fed with the same silage, and at the same age had to be pooled to make a good diagnostic. Some pictures are presented below as a visual illustration of the biopsy procedure.



Conclusion

It seems relevant to consider that a biopsy of fat (potentially completed by a blood sample) can be used in order to statute on the compliancy of the animal. This approach has then demonstrated its suitability in terms of evaluation of the residual contamination level in muscle, but most of all as extremely reactive tool in case of specific acute crisis situation. In our case older animals with levels above the limits were discarded from the commercial process, as well as younger ones presenting high level of contamination. For younger animals presenting an acceptable contamination level and still growing for a minimum of 4 to 6 months meaning animals able to increase significantly their weight and fat content, an additional control was organized.

Acknowledgements

We would like to thank the General Directorate for Food of the French Ministry of Agriculture (MAP, DGAl) for financial support, as well as the French Departmental Veterinary Services for technical cooperation.