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Predicting PCDD/F and dioxin-like PCB contamination levels in bovine edible tissues from *in vivo* sampling

Philippe Marchand [a], Ronan Cariou [a,*], Anaïs Vénisseau [a], Aline Brosseaud [a], Jean-Philippe Antignac [a,b], Bruno Le Bizec [a]

[a] ONIRIS, USC 2013, LABERCA, Atlanpole-La Chantrerie, BP 50707, Nantes F-44307, France
[b] INRA, Nantes F-44307, France

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ABSTRACT

European Union regulation has defined maximum levels for polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/F) and dioxin-like polychlorinated biphenyls (PCB) in food from animal origin. In case of particular event where livestock is potentially exposed to a specific source of contamination, the possibility of accurate estimation of the PCDD/F and dioxin-like PCB levels in edible tissues of the animals from *in vivo* and weakly invasive biological matrices (fast animal recovery, no major side effects) may be of clear valuable interest. This study investigated the correlations between contamination levels determined in subcutaneous adipose tissue and blood samples obtained from living animals and those measured after slaughtering in muscle and liver. The obtained results demonstrated very good correlations between these *in vivo* and *ex vivo* samples in terms of PCDD/F and PCB contamination levels. Finally, it seems to be demonstrated that a weakly invasive biopsy of subcutaneous adipose tissue performed on living animal (potentially completed by a blood sample) can be used in order to predict contamination levels in muscle and liver destined to human consumption.

1. Introduction

In spite of huge efforts completed for several years to reduce industrial emissions of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/F) and dioxin-like polychlorinated biphenyls (dl-PCB) in most of developed countries, some specific situations of acute contamination may still occur in food from animal origin. In most cases, the origin of such accidental events appears related with one or more feed's ingredients (ball clay, citrus pulp pellet, choline chloride and guar gum incidents for examples) or is due to the vicinity with plants industry releasing pollutants. The current European Union regulation regarding PCDD/F and dl-PCB in food is based on maximum tolerable limits established for various edible tissues or products (European Union Commission Regulation 1881/2006).

In case of incident involving contamination of livestock, farms located nearby the concerned area can be put into sequester, according to the precautionary principle. Once the contamination source is contained, authorities still have to statute on compliancy of products from a given farm. Apart from the investigation that can prove beyond doubt, accredited laboratories are often solicited. One drawback of the regulation is that such control of edible matrices is sometimes non-achievable from living animals. As typical example, the determination of PCDD/F and dl-PCB levels in muscle clearly always implies animal slaughtering. On the other hand, slaughtering animals or processing milk and eggs before being aware of analysis results appear inappropriate. In such context, useful tools to statute at an earlier stage would be welcomed.

For milk and eggs, sampling at the farm raises no obstacle. Therefore, main analytical concern is the rapidity of the results production. Current analytical processes usually require up to one week (confirmatory). For time and/or cost saving, screening approaches may be implemented, including bioanalytical techniques (Murk et al., 1996; Diaz-Ferrero et al., 1997; Vanderperren et al., 2004; Hoogenboom et al., 2006) or other emerging GC–MS related approaches (Eppe et al., 2004; Focant et al., 2005; Hoh et al., 2008; Malavia et al., 2008; Cariou et al., 2010). Once the result has been produced, there are then typically three possible scenarios. In the first one, the product is compliant and sequester can be lifted. In the second one, the contamination level is too high but given the fact that milk and eggs stand for routes of decontamination for the considered animal, a time period can be defined before product contamination becomes low enough to be sold on the market. Such decreasing decontamination kinetic studies has been published for time period decision but data remain scarce (Le Bizec et al., 2005; Brambilla et al., 2008). Anyway in this case, a new analysis control is needed. In the last scenario, the contamination

* Corresponding author. Tel.: +33 2 40 68 78 80; fax: +33 2 40 68 78 78.
E-mail address: laberca@oniris-nantes.fr (R. Cariou).

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level is also high but time required for decontamination is too long compared to economic losses: animals are discarded.

For meat or liver, sampling on live animal appears inappropriate, because of the physical and psychological trauma caused to the animal and to the farmer. Alternatively, predicting contamination levels from other types of tissues or fluids requiring weakly invasive sampling in accordance with basic animal welfare rules (fast animal recovery, no major side effects) would be acceptable. As persistent organic pollutants, PCDD/F and PCB are highly lipophilic, their distribution in various organism compartments is primarily related to fat content, implying that urine and hair are unfortunately not relevant as predicting matrices. Conversely, good candidates would be embodied by fatty matrices such as blood or subcutaneous fat biopsy. Yet, in such strategy, there is a clear lack of knowledge concerning relative contaminations between predicting and edible matrices, and influences of factors such as species, age and physiological state of the animal, feeding system or way of contamination. Finally, once the result has been produced, there are still three possibilities as previously described. Additionally to the second possibility, a time period can also be defined for non-lactating/non-laying animals (including males – no ability to excrete the pollutants through a biological route) to reach a compliant residual contamination merely by dilution in fat fraction when animal is growing. This dilution phenomenon has already been observed in pig, broiler or beef cattle (Thorpe et al., 2001; Hoogenboom et al., 2004; Spitaler et al., 2005). Tables reporting mass and fat increase of various species submitted to a given production system are available (Robelin, 1990; Micol et al., 1993; Diaz et al., 2001).

The aim of the present study was to investigate the correlations between predictive samples obtained from a living animal (blood and subcutaneous adipose tissue) and edible parts of this animal available after slaughtering (muscle and liver), with final scope to determine in what extents it would be possible to statute on the regulatory compliancy of the animal before the slaughtering. This work was based on data were obtained through two particular events that have occurred in France in 2006 and 2007, related to dl-PCB and PCDD/F exposures, respectively. In the latter case, the predicting model has been used in each sequestered farm from the contamination area regarding its sanitary status.

2. Material and methods

2.1. Samples

The first real case study included seven castrated male bovines between 3 and 4 years old originated from a single farm, and which were particularly exposed to dl-PCB through contaminated hay. All analyzed biological samples were collected after slaughtering. Adipose tissue samples included internal kidney fat ($n = 7$) and intraperitoneal fat ($n = 4$), as well as subcutaneous fat from behind the ear ($n = 7$) and from the neck ($n = 4$). Muscle samples were from the prime cut (thick skirt or hanging tender) ($n = 7$), the neck ($n = 4$), the shoulder ($n = 4$) and the topside (outside flat or bottom round) ($n = 7$). Serum ($n = 7$) and liver samples ($n = 4$) were also collected.

The second real case study included 15 castrated male bovines between 15 and 18 months old, originated from nine farms (no more than two animals per farm), and which were particularly exposed to PCDD/F through contaminated corn silage. Again, all analyzed biological samples were collected after slaughtering. Adipose tissue samples included internal kidney fat ($n = 15$) and subcutaneous fat from behind the ear ($n = 7$), from the sternum ($n = 7$) and from the sub-caudal areas ($n = 10$). Muscle samples were from the prime cut (thick skirt or hanging tender) ($n = 5$), the neck ($n = 10$), and the topside (outside flat or bottom round) ($n = 10$). Serum ($n = 14$) and liver samples ($n = 10$) were also collected.

All samples have been analyzed by the French National Reference Laboratory for PCBs and PCDD/Fs in food and feed, using the method described below that was accredited according to the ISO 17025 standard.

2.2. Subcutaneous biopsy

Subcutaneous biopsies were carried out by a veterinarian after local anaesthesia, according to current clinical good practice and basic animal welfare rules. After shaving and sanitization, a 2 cm incision was performed. Then, a subcutaneous fat sample (around 0.5 g) was collected. Finally, the incision was sutured and the animal remained under observation for a couple of hours. This whole sampling procedure was realised within no more than 10 min.

2.3. Sample extraction and clean-up

Details of the sample preparation method for fat, muscle and liver can be found elsewhere (Laurent et al., 2005; Antignac et al., 2006). Briefly, muscle and liver tissues were freeze-dried and powdered. Extraction of these two matrix types and adipose tissue was performed using a Pressurized Liquid Extraction system (ASE, Dionex, Sunnyvale, CA, USA). Three successive static extraction cycles (5 min each) were performed using a mixture of toluene/acetone (70:30, v/v) at 100 bar and 120 °C. Extracted lipids were dried overnight at 105 °C and determined gravimetrically. Serum samples (15 g) were diluted with saturated ammonium sulphate solution and ethanol before a two times extraction with hexane. Clean-up and fractionation of PCDD/F, non-ortho PCB and mono-ortho PCB were carried out using classically used liquid chromatography columns with sulphuric acid silica, Florisil and carbon (Carbopack C) in that order.

2.4. Serum lipids determination

Serum total lipids (TL) were determined using enzymatic dosages, for total cholesterol (TC) (Elical2, Kitvia, Labarthe-Inard, France), free cholesterol (FC) (Cholesterol-C, Wako Chemicals, Neuss, Germany), triglycerides (TG) (Triglycerides-LQ, Kitvia) and phospholipids (PL) (Phospholipids B, Wako Chemicals). Usually, for studies on human species, the Eq. (1) is used. The 1.677 factor corresponds to the ratio between weighted mean cholesterol ester molecular weight and cholesterol molecular weight. Used fatty acid composition of cholesterol esters was based on 24 healthy volunteers (Akins et al., 1989). To our knowledge, no equivalent factor has been calculated for bovine species in previous studies. Based on the study of Scislowski et al. (2004), the fatty acid composition of cholesteryl esters and triglycerides fraction in steers control group ($n = 6$) led us to a factor of 1.57 for bovine species.

$$TL = F^{*}(TC - FC) + FC + TG + PL \quad (1)$$

where $F = 1.677$ for human species (Akins et al., 1989), $F = 1.57$ for bovine species (data: Scislowski et al., 2004, calculation: present study).

2.5. GC–HRMS measurements

All samples have been analyzed by gas chromatography coupled to high resolution mass spectrometry (GC–HRMS). Identification and quantification of all target compounds (17 PCDD/F, 12 dl-PCB, 7 marker PCB) was achieved using the isotope-dilution method. ^{13}C-labeled internal standards were added before extraction for serum samples and after extraction for other matrices. GC separation was performed on a DB-5MS capillary column