Food and Chemical Toxicology 50 (2012) 1413–1417

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Levels and congener profiles of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in sheep milk from an industrialised area of Sardinia, Italy

M.M. Storelli [a,*], C. Scarano [b], C. Spanu [b], E.P.L. De Santis [b], V.P. Busco [a], A. Storelli [a], G.O. Marcotrigiano [a]

[a] *Pharmacological–Biological Department, Chemistry and Biochemistry Section, Veterinary Medicine Faculty, University of Bari, Strada Prov. le per Casamassima Km 3, 70010 Valenzano (Ba), Italy*

[b] *Animal Biology Department, Inspection of Food of Animal Origin Section, Veterinary Medicine Faculty, University of Sassari, Via Vienna 2, 07100 Sassari (SS), Italy*

ARTICLE INFO

Article history:
Received 3 November 2011
Accepted 6 January 2012
Available online 26 January 2012

Keywords:
Sheep milk
PCDDs
PCDFs
dl-PCBs
ndl-PCBs
Italy

ABSTRACT

Concentrations of 7 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs) and 22 polychlorinated biphenyls (PCBs), including 12 dioxin like-PCBs (non- and mono-*ortho* PCBs) were measured in 80 sheep milk samples from farms located in an industrialized area of Sardinia, Italy. PCDDs and PCDFs mean concentrations were 2.45 and 3.69 pg g^{-1} fat basis, respectively. The mean dl-PCB concentration was 2.01 ng g^{-1} fat basis, while cumulative ndl-PCB levels ranged from 1.02 to 20.42, with a mean of 4.92 ng g^{-1} fat. The results expressed in pg WHO-TEQ/g fat showed that contamination level of milk was below the limit values for human consumption established by EC legislation. In the same way, all the investigated milk exhibited PCDD/Fs concentrations below EU action levels, while dl-PCBs concentrations exceeded the action level of 2.0 pg WHO-TEQ/g fat. These findings point to the need to continue to conduct general monitoring programmes, including also milk samples from areas not close to the contaminant-emitting industries, in order to better evaluate the impact of industrial activities on surrounding environment.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) commonly known as "dioxins", together with polychlorinated biphenyls (PCBs) constitute three classes of structurally related chlorinated aromatic hydrocarbons. Among all these chemicals, the most toxic is the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) which was included in the list of the human carcinogens from International Agency for Research on Cancer (IARC, 1997). Next to TCDD, the 2,3,7,8-substituted PCDDs and PCDFs, actually 7 and 10 congeners and 12 PCBs, the so-called "dioxin-like PCBs" (dl-PCBs) are considered toxic to the similar extent. Due to their high lipophilic character, these analytes bioaccumulate in animal and human adipose tissues, causing a wide variety of toxic responses that include immunotoxicity, carcinogenicity and adverse effects on reproduction, development and endocrine functions (Safe et al., 1985, 1986). The literature reports that the most important route for human exposure to these toxicants is food consumption contributing over 90% of total exposure (Liem et al., 2000). Milk and dairy products are one of the major foodstuffs of animal origin that largely contribute to the human exposure to these toxic chemicals. Sheep milk represents an important renewable source of raw material for food applications. Most sheep milk produced in the world is, in fact, processed into speciality cheeses, yoghurts and other dairy products. Sheep milk production in Italy is mainly situated in rural and mountainous areas where the production systems are interrelated with local tradition and local sheep breeds (de Rancourt et al., 2006). Sardinia, in particular, is the Italian region leader for sheep milk production having 41% of the national sheep population (de Rancourt et al., 2006). Consequently, dairy sheep industry is strongly concentrated in this island where some of the most famous Italian cheeses are produced and exported all over the world. This milk is more concentrated in proteins and sugars than other forms of milk and in addition, it contains about 6–7% of fats, approximately twice than that of cow milk, so it is may be especially prone to accumulate these lipophilic contaminants. In spite of this, the literature still has not many evaluation data of the contamination levels of sheep milk, especially for Italy (Ingelido et al., 2009; Esposito et al., 2010a), although it stands among the four main Mediterranean countries for sheep milk production (de Rancourt et al., 2006). To fill this knowledge gap, the present study determines the levels and congener profiles of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), including dioxin-like PCBs (dl-PCBs) in sheep milk from Sardinia (Italy) and ascertains whether the concentrations of these pollutants are below the limit values for

* Corresponding author. Tel.: +39 80 5443365; fax: +39 80 5443363.
E-mail address: m.m.storelli@veterinaria.uniba.it (M.M. Storelli).

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2012.01.008

human consumption established by European Commission legislation (Official Journal of the European Union, 2006a). This aspect is especially interesting because milk samples investigated come from farms located in an industrialized area of Sardinia, Italy. The measured levels will be also commented on the basis of available literature data on this issue.

2. Materials and methods

2.1. Sample collection

Between May and June 2009, 80 sheep milk samples were collected by qualified veterinary inspectors in 12 small farms located in different parts of the Cagliari province, in South-Eastern Sardinia (Italy) (Fig. 1). The farms designated for the milk sampling were located at different distance (from 7 to 23 km) from potential sources of contamination, including oil refinery, petrochemical industry, metal industry etc. Milk samples were taken when the animals are kept outdoors and their diet is based mainly on fresh grass. The milk samples (about 1000 mL) were stored in chemically clean glass recipients, frozen at −20 °C, and then shipped on dry ice to the laboratory for the analysis. Milk was sampled following the requirements of ISO/IEC 17025 (International Organization for Standardization (ISO), 2005) and in agreement with the European Commission Regulation 1883/2006 (Official Journal of the European Union, 2006b).

2.2. Sample preparation and clean up

The concentrations of 22 individual congeners (PCB 20, 28, 35, 52, 60, 101, 138, 153, 180 and 209), including the "dioxin-like" PCBs (dl-PCBs) (non-*ortho* PCBs: 77, 81, 126 and 169; mono-*ortho*: PCB, 105, 114, 118, 123, 156, 157, 167, 189), together with the seventeen 2,3,7,8-substituted PCDD/Fs (PCDDs: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD; PCDFs: 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF) were determined. The milk samples were subjected to liquid–liquid extraction with diethyl ether and petroleum ether (1:1) (Eljarrat et al., 2002). The extracts were concentrated and the fat content of milk was gravimetrically calculated. Fat extract was divided into two aliquots prior to purification for determination of PCDD/Fs and PCBs. In particular, for PCDD/Fs the US EPA Method 1613 (1994) was used. Briefly, the samples extracted as above reported have been subjected to a multi-step clean-up to remove the matrix and the potential interfering components. The first stage was a fat destruction step consisting of a treatment of the sample solution with sulfuric acid and base back-extraction. The obtained extracts were then subjected to a pre-conditioned florisil clean-up column, that was eluted with different solutions in order to remove interfering components. The first eluted solvent was discarded, while the second eluate which contained PCDD/Fs was collected. The extracts were evaporated to dryness and redissolved in iso-octane. Appropriate ^{13}C-labeled standards (EPA 1613LCS, EPA 1613CSS and EPA 1613ISS) (Wellington Laboratories, Guelph, Ontario, Canada) were used. Recoveries of the ^{13}C-labeled standards ranged from 89% to 101%. For PCBs determination the samples, after lipid removal with a sulphuric acid treatment, were cleaned by passing through 8 g of acid silica (H_2SO_4, 44% w.w.), using 50 mL of a mixture of hexane/dichloromethane (1/1, v/v) for elution of the analytes. The eluate was evaporated to dryness and redissolved in 100 μL of iso-octane. For the separation of non-*ortho* PCB congeners (no. 77, 126 and 169) from other PCBs, the method reported by Tanabe et al. (1987), involving fractionation on 125 mg of activated carbon (434455 C. Erba, Milano, Italy), was used. Congener PCB 143 (AccuStandard Inc., New Haven, CT, USA) was added as internal standard, and a mixture of ^{13}C-labeled dl-PCBs (MBP-CP Wellington Laboratories) was added as recovery standard before extraction (recovery >75%).

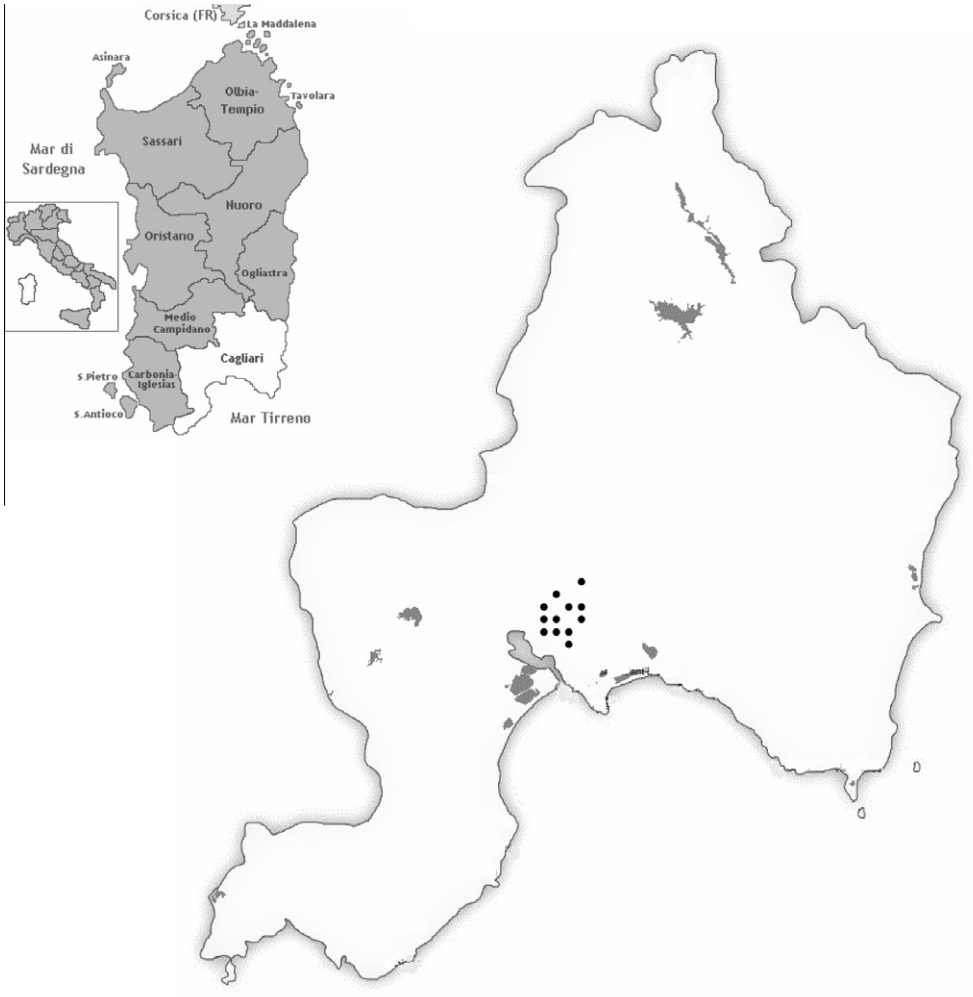

Fig. 1. Sampling area.