

## ORIGINAL ARTICLE

# Occurrence of polychlorobiphenyls in buffalo mozzarella cheese from Campania (Italy)

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## Key words

Polychlorobiphenyls • Buffalo milk • Buffalo mozzarella cheese

## Summary

Buffalo milk and mozzarella cheese produced in the Caserta and Salerno areas in Campania region have been investigated on the presence and the levels of polychlorobiphenyls (PCBs). Seven congeners, six non dioxin-like (NDL-PCBs nos. 28, 52, 101, 138, 153 and 180) and one dioxin-like (DL-PCB n. 118), were detected. PCBs were found at detectable levels in the 83% of the buffalo milk and in the 100% of the mozzarella cheese samples from Caserta; in those from Salerno the prevalence of contamination was 77% for milk and 73% for mozzarellas, respectively. The NDL-PCB content of mozzarellas collected in

Caserta was significantly higher than that found in those from Salerno. The more diffuse congeners were PCB 28, 138 and 153 both in milk and in mozzarella cheese; PCB 118 contributed to the total PCB content for the 7% in milk and 2-3% in mozzarella cheese. On the basis of the Italian annual average consumption the contribution of mozzarella to the daily dietary intake of NDL-PCB can vary between 0.41 and 21.33 ng kg<sup>-1</sup> bw, median value of 3.66 ng kg<sup>-1</sup> bw. The levels of contamination in milk and dairies analyzed are similar or quite lower than those found in other European countries.

## Introduction

The presence of chemical pollutants in foods is an important item on the food safety.

Among xenobiotics notable interest is aroused on chemical halogenated compounds as dioxins, dibenzofurans and polychlorobiphenyls (PCBs). PCBs are ubiquitous in environment and because of their persistence and accumulation in the food chain, food consumption is the major pathway of human exposure accounting for > 90% if compared to other ways of exposure such as inhalation and dermal contact. The total dietary PCB intake by the general population depends greatly on the geographical area and food habits; in various European countries milk and dairy products are the major contributors [1]. Although levels of PCBs in food have gradually decreased since environmental legislation on their use and disposal was introduced, human exposure to PCBs is considered to be still high [2].

On the basis of their structures and toxicological properties, among PCBs two groups, the dioxin-like (DL-PCBs) and the non dioxin-like (NDL-PCB) polychlorobiphenyls are distinguished. For their ubiquity and frequency of availability, seven polychlorobiphenyl congeners, six non dioxin-like (IUPAC nos. 28, 52, 101, 138, 153 and 180) and one dioxin-like (IUPAC n. 118), are generally assessed as PCB contamination markers in biological and environmental matrices.

International literature reports many toxicological data on DL-PCBs which can be used for the assessment of health risks, but in contrast very little toxicological information are available on NDL-PCBs and their tox-

icity profiles. The European Commission has expressed concern that NDL-PCBs can cause neurobehavioral, reproductive and peri-natal development problems, as well as promote tumours. The adverse effects reported in laboratory animals following exposure to individual NDL-PCB were effects on the thyroid, liver and brain biochemistry, as well as immunotoxicity, oestrogenicity, and reproductive and neurodevelopmental effects. Since NDL-PCBs constitute the major part of the PCBs found in food and human tissues [2], to investigate on their presence and concentration in foods is essential to adopt precautionary actions to protect consumer's health.

Dairy products represent an important component in the European diet.

Studying the level and sources of PCBs Zuccato et al. [3] reported that the dietary exposure to total PCBs in the Italian diet was mainly attributable to dairy products. As in other European countries, as France and the Netherlands, in Italy many kinds of traditional cheeses are produced. Among these the buffalo mozzarella cheese is largely consumed in Italy and mainly in Campania region that represents the typical production zone. From 1996 the buffalo mozzarella produced in Campania was recognized as a "DOP" (Denomination of Protected Origin) cheese by CEE [4]. Its production is regulated by the Italian DPR (28/09/1979) which establishes that buffalo mozzarella cheese produced as "DOP mozzarella di bufala campana" must be made exclusively from milk of buffalos leaving in zones tightly defined. The provinces of Caserta and Salerno in Campania region are the typical zones of buffalos' rearing and, consequently, the

dairy farms are mainly concentrated in the same zones. In the province of Caserta, some dairy farms, none of which among the DOP “*mozzarella di bufala campana*” producers, have been recently interested in dioxin pollution of milk and mozzarella cheese at levels quite higher than the limits set from the EU, but data about the PCB pollution are not available.

The objective of the present study has been to investigate on the presence and the levels of PCBs in buffalo milk and mozzarella cheese produced in the Caserta and Salerno areas.

## Materials and method

### SAMPLING

In the period February 2007-March 2008 all the dairy farms producers of buffalo mozzarella in the provinces of Caserta and Salerno were visited and the aim of the study was descript. For each province fifteen farms, all DOP “*mozzarella di bufala campana*” producers, agreed to be involved in the study. In every farm buffalo milk and mozzarella cheese were sampled randomly during a week. Mass milk and cheese samples were put in glass pots and refrigerated until coming in laboratory. Here, milk was divided in 10 ml aliquots and lyophilized; cheese was homogenized and then divided in 5 g aliquots that have been lyophilized too.

### PCB ANALYSIS

NDL-PCBs nos. 28, 52, 101, 138, 153 and 180 and the DL-PCB n. 118 were determined using the “Istituto Superiore di Sanità” analytical method [5] with some revisions. From lyophilized samples fat was extracted three times by cold light petroleum + acetone (50:50 v: v), the extracts centrifuged at 1600 r.p.m. for 15 min and concentrated under vacuum at 40°C. The fat extracted was quantified gravimetrically. The lipidic dried extracts were dissolved in n-hexane, transferred on a system Extrelut-3/Extrelut-1 cartridges (Merck Kga A Darmstad, Germany), and eluted with acetonitrile. The eluates were concentrated at 1 ml under vacuum at 40°C and then poured into a glass column containing 2.5 g of Florisil (60/100 mesh – Supelco Bellefonte, PA, USA) activated overnight at 130°C; the column was eluted three times with 10 ml aliquots of n-hexane collecting the eluates. Gas chromatographic analyses of the PCBs were carried out by a HEWLETT – PACKARD 5890 Series III gas chromatograph with Electron Capture Detector (ECD) injecting 1 µl of the extracts on a HP-5 (Crosslinked 5% PH ME Siloxane, 30 m length, 0.32 i.d., 0.25 µm film thickness) glass capillary column. Helium and Argon methane were respectively used as carrier and make-up gases. Injector temperature was set at 300°C and ECD at 320°C. The analysis was carried out with a temperature program 60°C for 2 min, increasing of 10°C/min to 170°C, increasing of 2°C/min to 210°C, increasing for 15°C/min to 260°C staying at this temperature for 20 min.

PCBs were identified and quantified by comparing the retention time and the area of each individually resolved peak with that of the corresponding reference standard. PCB 209 was used as internal standard. Reference standards were obtained by Dr. Ehrenstorfer GmbH Labservice Analytica S.r.l. Anzola Emilia, Bologna, Italy.

Detection limits (LOD) for PCBs were 0.09 ng g<sup>-1</sup> fat weight (fw) for PCBs 52, 101, 138, 153, 180 and 0.60 ng g<sup>-1</sup> fw for PCBs 28 and 118. Quantification limits (LOQ) were 0.10 ng g<sup>-1</sup> fw for PCBs 52, 101, 138, 153, 180 and 0.85 ng g<sup>-1</sup> fw for PCBs 28 and 118. The results were expressed as ng g<sup>-1</sup> on fat (fw) and wet weight (ww); they are referred as not detectable (n.d.) when the concentrations were lower than the LODs. Accuracy and reproducibility were established by a regular quality control procedure which includes all the analytical series to be accompanied by control samples and reactive blanks.

The recoveries, tested by extracting samples fortified with a known amount of standard mixtures at three concentration levels analyzed in triplicate, ranged between 80 ± 3% (PCB 28, 52, 118) and 90 ± 4% (PCB 101, 138, 153, 180). The total amount of the PCBs determined was calculated as the sum of the concentrations of the seven indicator congeners (Σ PCBs) and of the six NDL-PCBs (Σ NDL-PCBs).

### STATISTICAL METHODS AND ANALYSES

Statistical data analysis was performed with SPSS 13.0. The significativity of the differences in concentrations of PCB in milk and cheese among the production zones were evaluated by one-way analysis of variance by the (ANOVA) Tukey Multiple Comparisons Test, assuming for individual PCB that, when a result was below the detection limit (n.d.), the value was one-half of the LOQ. The level of significance was set at p < 0.05.

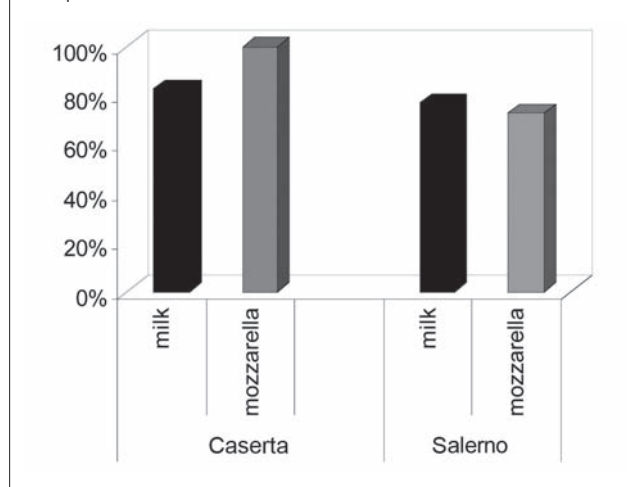
## Results and discussion

The milk samples from Caserta and Salerno presented respectively 24.00 ± 2.15 and 25.80 ± 1.81 g% of fat which correspond to 53 and 51 g% of fat on dry substance. These contents are in according with the Italian DPR (n. 28/09/1979) which establishes that the buffalo mozzarella cheese must contain a minimum fat content of 52 g% (on dry weight) and a maximum humidity of 65 g%.

PCBs were found at detectable levels in the 83% of the buffalo milk and 100% of the mozzarella cheese samples from Caserta; in those from Salerno the prevalence of contamination was 77% for milk and 73% for mozzarellas, respectively (Fig. 1).

PCB concentrations in milk and cheese are shown in Table I. In milk samples from Caserta the total contents of the PCBs analyzed ranged between n.d. and 43.71 ng g<sup>-1</sup> fw, median 12.75 ng g<sup>-1</sup> fw; in those from Salerno they varied from n.d. to 34.00 ng g<sup>-1</sup> fw, with a median of 13.55 ng g<sup>-1</sup> fw. The median concentration amounted to 30.61 ng g<sup>-1</sup> fw (range 5.88-155.16 ng g<sup>-1</sup> fw) in the mozzarella samples collected in the dairy farms from Caserta

**Fig. 1.** Contamination prevalence of PCBs in milk and mozzarella cheese produced in the Caserta and Salerno provinces in Campania.



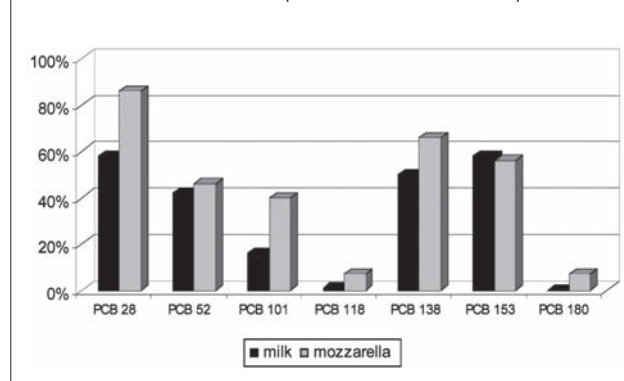
province, while in those from Salerno the median was  $9.66 \text{ ng g}^{-1} \text{ fw}$  (range n.d.- $67.75 \text{ ng g}^{-1} \text{ fw}$ ). Unfortunately during the sampling it was not possible to follow everytime the whole productive cycle, from milk to cheese, collecting the milk immediately before its employing and this can probably explain why a correspondence between the PCB contents of milk and those of cheese was not found. In milk from Caserta and Salerno the NDL-PCB concentrations varied from  $4.08$  to  $43.00 \text{ ng g}^{-1} \text{ fw}$  (median  $11.85 \text{ ng g}^{-1} \text{ fw}$ ) and from  $3.39$  to  $29.00 \text{ ng g}^{-1} \text{ fw}$  (median  $11.57 \text{ ng g}^{-1} \text{ fw}$ ), respectively. In the mozzarella samples collected in the dairy-farms from Caserta province, the median concentration amounted to  $25.62 \text{ ng g}^{-1} \text{ fw}$  (range  $5.45$ - $155.16 \text{ ng g}^{-1} \text{ fw}$ ), while in those from Salerno the median was  $9.32 \text{ ng g}^{-1} \text{ fw}$  (range  $1.39$ - $67.45 \text{ ng g}^{-1} \text{ fw}$ ). No significant differences in the PCB pollution levels were found in milk from the two zones, while the PCB contents in mozzarellas collected

in Caserta were significantly higher ( $p = 0.015$ ) than in those from Salerno.

The prevalence of contamination and the concentrations of individual PCB congeners in samples from Caserta are listed in Figures 2 and 3. The more diffuse congeners were PCB 28, 138 and 153 both in milk and in mozzarella cheese. In mozzarella cheese PCB 28 mean level ( $10.63 \text{ ng g}^{-1} \text{ fw}$ ) was about one order of magnitude higher than those of PCB 138 and 153.

In the samples coming from Salerno the most representative congeners were PCB 138, found in the 69% of milk and the 60% of mozzarella samples, and PCB 153, detected in the 69% of milk and the 50% of mozzarella samples, at median concentrations, respectively, for PCB 138, of  $1.49 \text{ ng g}^{-1} \text{ fw}$  in milk and  $1.16 \text{ ng g}^{-1} \text{ fw}$  in mozzarella cheese, and, for PCB 153, of  $1.34 \text{ ng g}^{-1} \text{ fw}$  in milk and  $1.31 \text{ ng g}^{-1} \text{ fw}$  in mozzarella cheese. PCB 28 was detected in the 8% of the milk samples and in the 40% of the mozzarella samples, with median values of  $0.85 \text{ ng g}^{-1} \text{ fw}$  in milk and  $0.35 \text{ ng g}^{-1} \text{ fw}$  in mozzarella cheese. The congeners PCB 118 and 180 were found the first, in the 30% of the milk (median  $0.96 \text{ ng g}^{-1} \text{ fw}$ ) and

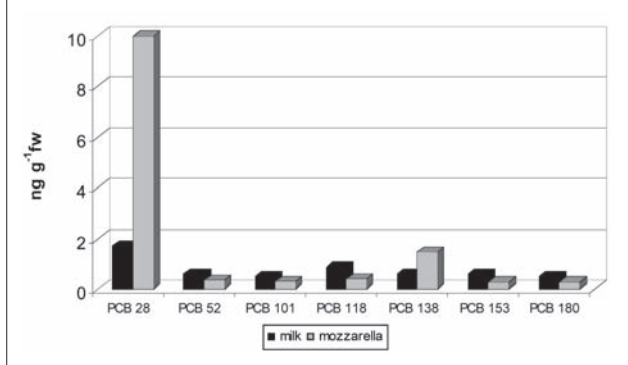
**Fig. 2.** Contamination prevalence of individual PCB congeners in milk and mozzarella cheese produced in the Caserta province.



**Tab. I.** PCB contamination levels ( $\text{ng g}^{-1}$  on wet and fat weight) in milk and mozzarella cheese produced in the Caserta and Salerno provinces in Campania.

Origin zone	Product	$\Sigma$ PCBs				$\Sigma$ NDL-PCBs			
		$\text{ng g}^{-1} \text{ ww}$		$\text{ng g}^{-1} \text{ fw}$		$\text{ng g}^{-1} \text{ ww}$		$\text{ng g}^{-1} \text{ fw}$	
		Mean $\pm$ S.D. (Range)	Median	Mean $\pm$ S.D. (Range)	Median	Mean $\pm$ S.D. (Range)	Median	Mean $\pm$ S.D. (Range)	Median
Caserta	Milk	$0.97 \pm 0.83$ (n.d. - 3.06)	0.77	$16.21 \pm 12.21$ (n.d. - 43.71)	12.75	$0.93 \pm 0.83$ (0.20-3.01)	0.72	$15.37 \pm 12.31$ (4.08-43.00)	11.85
	Mozzarella cheese	$8.89 \pm 6.59$ (1.41-23.37)	6.25	$42.28 \pm 38.61$ (5.87-155.16)	30.61	$8.71 \pm 6.64$ (1.31-23.27)	6.15	$41.38 \pm 38.74$ (5.45-155.16)	25.62
Salerno	Milk	$0.69 \pm 0.39$ (n.d. - 1.28)	0.68	$13.55 \pm 9.24$ (n.d. - 34.00)	13.55	$0.61 \pm 0.36$ (0.20-1.23)	0.58	$11.82 \pm 8.39$ (3.39-29.00)	11.57
	Mozzarella cheese	$4.18 \pm 5.67$ (0.55-22.56)	2.82	$13.48 \pm 17.34$ (n.d. - 67.75)	9.66	$3.99 \pm 5.68$ (0.45-22.46)	2.72	$12.87 \pm 17.37$ (1.39-67.45)	9.32
Total (Caserta and Salerno)	Milk	$0.83 \pm 0.64$ (0.25-3.06)	0.68	$14.82 \pm 10.62$ (4.23-43.71)	13.54	$0.76 \pm 0.64$ (0.20-3.01)	0.58	$13.53 \pm 10.39$ (3.40-43.00)	11.57
	Mozzarella cheese	$6.53 \pm 6.49$ (0.55-23.37)	4.42	$27.88 \pm 32.85$ (1.71-155.83)	15.10	$6.35 \pm 6.53$ (0.45-23.27)	4.00	$27.12 \pm 32.86$ (1.40-155.16)	14.50

Fig. 3. Median contamination levels of individual PCB congeners ( $\text{ng g}^{-1}$  fat weight) in milk and mozzarella cheese produced in the Caserta province.



in the 6% of the mozzarella samples (median  $0.34 \text{ ng g}^{-1}$  fw), the latter, in the 54% of the milk and 26% of the mozzarella samples (medians, respectively,  $0.77 \text{ ng g}^{-1}$  fw and  $0.24 \text{ ng g}^{-1}$  fw) (Figs. 4 and 5).

The most representative congeners both in milk and cheese were PCB 138 and PCB 153; these hexa-chloro biphenyls congeners are reported to be the prevalent compounds in biological samples for their high degree

Fig. 4. Contamination prevalence of individual PCB congeners in milk and mozzarella cheese produced in the Salerno province.

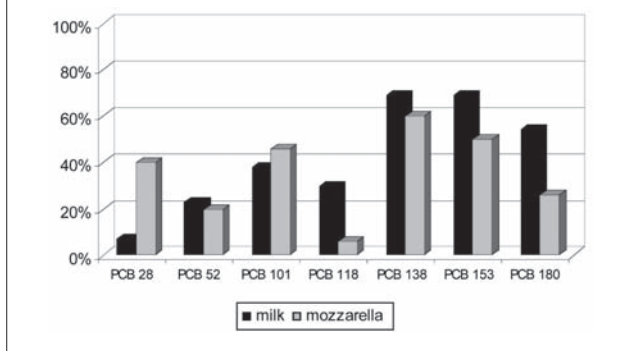
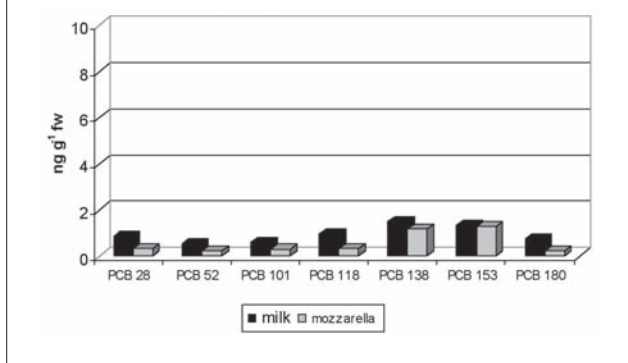


Fig. 5. Median contamination levels of individual PCB congeners ( $\text{ng g}^{-1}$  fat weight) in milk and mozzarella cheese produced in the Salerno province.



of chlorination. Because of the lack of unsubstituted ring positions on their biphenyl rings available for enzymatic attack the metabolic degradation by animal organisms is difficult. Consequently, they are eliminated more slowly and some compounds, such as PCB 138, 153, 118 and 180, appear to be transferred unchanged in milk. Vrecl et al. [6] observed that compound planarity and lipophilicity may influence the milk excretion of PCBs, so, generally, the lower chlorinated congeners are metabolised and much less excreted in milk than the higher ones. They are in fact absorbed within the digestive tract and combined with the lipid components of the blood and circulate through the body; finally they are metabolised in the liver by enzymes [2]. Furthermore it was attested that the key of PCB persistence in the animal organisms is the chlorine substitution in the 4 and 4' positions [7] that are the positions displayed by the above mentioned congeners. Moreover in our samples PCB 28, a tri-chlorinated congener, was quite present too, mainly in milk and mozzarella cheese from Caserta, in which contributed, respectively, for the 13% and the 56% to the total PCB content. PCB 118 contributed to the total PCB content for the 7% in milk and 2% in mozzarella samples from Caserta and for the 7% in milk and 3% in mozzarella samples from Salerno. PCB 118 is a dioxin-like PCB, to which a TEF value (Toxicity Equivalent Factor) of 0.00003 was attributed [8]. Considering the median concentrations of PCB 118 in the samples from the two zones, the relative TEQs were calculated. Mean values of 0.029 and  $0.013 \text{ pg TEQ/g fat}$  respectively for milk and mozzarellas from Caserta, and of 0.032 and  $0.010 \text{ pg TEQ/g fat}$  respectively for milk and mozzarellas from Salerno were obtained. These values are one order of magnitude higher than those found by Santelli et al. [9] on analogous products on sale in Caserta and Reggio Calabria supermarkets ( $\text{TEQ mean values of } 0.002 \text{ pg TEQ/g fat}$  in buffalo milk and  $0.002 \text{ pg TEQ/g fat}$  in mozzarella cheese). Since in this study among the DL-PCBs only the PCB n.118 was determined, the evaluation of the risk assessment for this group of pollutants isn't possible.

The concentrations of PCBs found in all the milk and dairy samples analyzed are lower than the limit proposed by 1999/449/EC Directive [10] ( $100 \text{ ng g}^{-1}$  fw) with the exception of one mozzarella sample from Caserta in which PCB concentrations amounted to  $155.16 \text{ ng g}^{-1}$  fw. They are also noticeably lower than the FDA tolerance values for PCB residues in milk and manufactured dairy products established in the USA by the FDA ( $1.5 \text{ ppm}$  on fat basis).

Sewart and Jones [11] studying PCB contamination in UK cow's milk found that the dominating congeners were PCB 118, 153, 138 and 180, whose mean concentrations amounted to  $1.24 \text{ ng g}^{-1}$  fw for PCB 118;  $1.67 \text{ ng g}^{-1}$  fw for PCB 153;  $1.43 \text{ ng g}^{-1}$  fw for PCB 138;  $0.72 \text{ ng g}^{-1}$  fw for PCB 180, results quite similar to those found in the present study.

About PCB 28, our finding in products from Caserta is anomalous. No similar data are described in scientific literature. So further studies need to explain why this

congener was so diffuse and the level of contamination so high in dairies from that zone.

Dietary exposure to PCBs was studied in Italy in the mid-1990s [3]. The method was based on collection of duplicate meals, on two non-consecutive days, in a group of twenty subjects consuming a typical central Italian diet. For the sum of the seven indicator PCBs a mean daily intake of 19 ng kg<sup>-1</sup> bw, with a 95th percentile of 40 ng kg<sup>-1</sup> bw was calculated. The mean and the 95th percentile values for the six indicator NDL-PCBs were 18 and the 39 ng kg<sup>-1</sup> bw per day, respectively. The dietary exposure to total PCBs was mainly attributable to dairy products. PCBs 153 and 138 were the most abundant congeners as reported in our study too.

Pinelli et al. [12] analyzed milk samples from seven farms in North Italy confirming that the seven indicator PCBs represented more than the 90% of all the PCBs found in mass milk of North Italy.

In order to assess the occurrence of NDL-PCBs in food and feed in Europe the EFSA collected values for dairy products by 15 Member States [13, 14]. The highest median values were referred for PCB 138 (2.60 ng g<sup>-1</sup> fw) and PCB 153 (3.10 ng g<sup>-1</sup> fw), while median values were 1.50 ng g<sup>-1</sup> fw for PCB 28 and 180, 1.00 ng g<sup>-1</sup> fw for PCB 52 and 118 and 1.10 ng g<sup>-1</sup> fw for PCB 101. The median values obtained in the present study on buffalo mozzarella cheese were lower or similar (PCB 28 in mozzarella from Caserta at concentration of 1.72 ng g<sup>-1</sup> fw).

Since, as underlined above, NDL-PCBs constitute a major part of the PCBs found in food and human tissues the assessment of their adverse effects on health needs to be urgently done. The European Commission has asked the Scientific Committee of Food to make a study to evaluate their toxicity in man and to identify the main food sources and their relative importance in the diet. Also, considering that the current calculation of TEQ is made only by the sum of the dioxin and the dioxin-like PCBs, it is trying to revalue the concept inserting in the calculation the "additive dose" too, made from the contribution of compounds different from the dioxin, as the NDL-PCBs (EFSA Scientific Colloquium 2004). In fact, samples containing high levels of NDL-PCBs will usually also contain high levels of DL-PCBs and PCDDs and PCDFs. In these circumstances, risk management measures to reduce DL-PCB TEQ and total TEQ levels will probably also protect consumers from elevated NDL-PCB exposure [2]. To provide regulators with a health-based guidelines to prevent adverse health effects of exposure to indicator PCBs is recommended the estimation of a Tolerable Daily Intake and, since a

high intake of PCBs is usually accompanied by an intake of dioxin, monitoring of the dietary intake of PCBs is just as important as that of dioxin.

The EFSA estimated recently an average intake of NDL-PCBs (sum of six congeners) of 10-45 ng kg<sup>-1</sup> bw for the general EU adult population. No data are available on young children, but, because of the higher food consumption in relation to the body weight, a pollutant exposure on bodyweight basis higher in children than in adults is expected.

Unfortunately data on the mozzarella consumption by the Campania people are not available, but, considering the average consumption of mozzarella (both from cow and buffalo milk) in Italian people (20 kg/year) and the values obtained in this study for the NDL-PCB concentrations in mozzarella cheese, ranging from 0.45 to 23.27 ng g<sup>-1</sup> ww, median 4.00 ng g<sup>-1</sup> ww, we estimated that for adults of 60 kg bw the mozzarella contribution to the average daily dietary intake of NDL-PCB can be between 0.41 and 21.33 ng kg<sup>-1</sup> bw, with a median value of 3.66 ng kg<sup>-1</sup> bw. For the sum of the seven indicator PCBs our data showed a daily intake between 0.60 and 25.50 ng g<sup>-1</sup> ww, with a median value of 4.82 ng kg<sup>-1</sup> bw. Because the consumption data are referred to cow and buffalo mozzarellas, the contribution of the buffalo mozzarella to the total PCB exposure would meanly result lower.

The results of this study confirm a wide diffusion of PCBs in the milk and dairies analyzed that show levels of contamination similar or quite lower than those referred to European countries by the EFSA.

On the basis of these considerations we can conclude that the gaining of scientific knowledges on the adverse effects of the considered pollutants on children and young people health and on possible synergic actions adverse to the human health developed by food pollutants will can allow to define specific safety dietary guidelines. It is very important to provide a scientific basis for defining risk standards on NDL-PCBs but also to lower the levels of NDL-PCBs in food by continuing to control their release into the environment, as recommended by the EFSA. In foods of animal origin an active surveillance on the feed contamination must be done, setting specific limits for NDL-PCBs, since from 2002 the maximum permitted levels for a range of contaminants as dioxins, organochlorine pesticides, heavy metals, etc., in animal feedstuffs have been set by the EU Directives 2002/32/EC and 2006/77/EC, but currently no specific limits are available for NDL-PCBs.

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