From environment to food: the case of PCB

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Summary. Polychlorinated biphenyls (PCB) are ubiquitary microcontaminants. Because of both lipid solubility and the absence of adeguate metabolic pathway in the organisms, PCB tend to bioaccumulate along the trophic chains. PCB may affect the endocrine, nervous and immune systems; the biological activities are related to chemical structure, particular concern has arisen about a group of "dioxin-like" congeners. Feed is the major way of PCB exposure of farm animals. PCB bioaccumulation is related to the lipid content of tissues, particularly in fat tissues, in meat, liver and are transferred into milk and eggs. More than 90% of human exposure derives from foods of animal origin. A recent episode of PCB contaminating pastures occurring in the industrial municipality of Brescia (Northern Italy) is presented as an example of feed-food-human transfer chain. Farm animals and human exposure to dioxin-like and non dioxin-like PCB congeners is discussed in relationship to their toxicological effects, with special attention to vulnerable groups, such as children. Finally, considerations on risk assessment and management are reported.

Key words: food, environment, risk, health, animals, chemistry.

Riassunto *(Dall'ambiente agli alimenti: il caso dei PCB)*. I policlororo bifenili (PCB) sono contaminanti ubiquitari. A causa della loro lipofilicità e dell'assenza di un adeguato metabolismo i PCB bioaccumulano negli organismi lungo la catena trofica. I PCB interferiscono con i sistemi endocrino, nervoso ed immunitario in relazione con la struttura chimica; ad esempio, particolare preoccupazione desta il gruppo di congeneri "diossina-simili". Il maggior apporto di PCB nella dieta umana è a carico degli alimenti di origine animale. Per gli animali d'allevamento i mangimi sono la maggior via di esposizione a PCB, accumulandosi nei tessuti adiposi, nelle carni, nel fegato, nelle uova e nel latte. Il recente episodio di contaminazione da PCB dei pascoli verificatosi a Brescia, rappresenta un esempio di trasferimento dall'ambiente agli alimenti zootecnici all'essere umano. L'esposizione di animali d'allevamento e dell'uomo ai congeneri di PCB diossina-simili e non diossina-simili è esaminata anche in relazione ai diversi effetti tossicologici, in particolare riguardo a gruppi vulnerabili, quali i bambini. Infine vengono riportate considerazioni sulla valutazione e gestione del rischio.

Parole chiave: alimenti, ambiente, rischio, salute, animali, chimica.

INTRODUCTION

Polychlorinated biphenyls (PCB) cover a group of 209 different congeners, depending on the number and the position of chlorine atom substituents. Due to their non-flammability, chemical stability and high dielectric constants, technical PCB mixtures have been widely used in a number of industrial and commercial applications, including fluids in transformers, capacitors and hydraulic transfer systems, plasticizers in paints, dyes, plastics and rubber products, etc. [1]. It is estimated that more than 1 million tons of technical PCB mixtures were produced world-wide since their first commercial use in the late 1920s. However, since the 60s concerns have grown about the potential for environmental persistence, and bioaccumulation as well as , the potential long-term effects on endocrine immune and nervous systems of different living organisms. Accordingly, the manufacture, processing and distribution of PCB has been prohibited in almost all industrial countries since the late 1980s [2]; however their entry into the environment still occurs, especially due to improper disposal practices or leaks in electrical equipment and hydraulic systems still in use [3, 4].

Most important PCB are highly persistent and are globally circulated by atmospheric transport and thus are present in all environmental media; because of both lipid solubility and the absence of adeguate metabolic pathway in the organisms the PCB tend to bioaccumulate along the trophic chains. As a consequence, PCB as a whole are major components of "persistent organic pollutants" (POPs) together with polychlorodibenzodioxins and polychlorodibenzofurans (PCDD/Fs) [5].

EXPOSURE

PCB congeners can be divided into two groups according to their toxicological properties, that, in turn, are relevant to exposure assessment.

One group, consisting of 12 congeners, shows toxicological properties similar to PCDD/F and is therefore termed "dioxin-like PCB" (DL-PCB). Alike PCDD/F DL-PCB are agonists of aryl hydrocarbon receptor

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(AhR) [6] and exposure has to be assessed together, since in almost all matrices, both groups of chemicals are not found as single compounds but as complex mixtures [7]. Obviously the contamination pathways may be different, as PCDD/PCDF are still actively introduced in the environment as combustion products.

The combined exposure to DL-PCB and PCDD/F is expressed as toxic equivalents (TEQ). The conversion of analytical results into TEQ is based on the assumption that PCDD/F and DL-PCB share the mechanism of action and cellular targets, albeit with different potencies. These differences in toxicity are expressed in the toxic equivalency factors (TEFs), estimated for each congener in relation to the most toxic congener 2,3,7,8-TCDD (the "Seveso" dioxin), which is assigned the arbitrary TEF of 1 [8].

By multiplying the analytically determined amounts of each congener by the corresponding TEF and summing the contribution from each congener the total TEQ value of a sample can be obtained using the following equation:

$$TEQ = (PCDDi \times TEFi) + (PCDFi \times TEFi) + (PCBi \times TEFi)$$

The most widely used schemes have been the International TEFs (I-TEFs) (NATO/CCMS, 1988) for PCDDs and PCDFs and the WHO-TEF included the DL-PCB for both human (WHO-TEFs) and fish and wildlife risk assessment [9].

TEFs for dioxin-like compounds apply only to AhR-mediated responses considered as the critical effect for hazard assessment. The criteria used by WHO for including a compound in the TEF scheme for dioxin-like compounds were that the compound must: a) show a structural relationship to the PCDDs and PCDFs, b) bind to the AhR, elicit AhR receptor-mediated biochemical and toxic responses, and c) be persistent and accumulate in the food chain.

The use of this concept assumes dose additivity. While additivity predominates in the majority of experimental studies, non-additive interactions of PCDD, PCDF and PCB mixtures, in particular antagonistic effects, have been reported at greater than environmental levels of exposure. These non-additive effects are considered to be due to multiple mechanisms of action of individual congeners and/or to pharmacokinetic interactions [10].

Non dioxin-like PCB (NDL-PCB) comprise the vast majority of congeners of the PCB residues in the feed-food chain. Most NDL-PCB have been shown to interact with endocrine, cytokine and/or neurotransmitter networks; these compounds should not be considered as an undifferentiated lot since there are differences in the persistence, bioaccumulation and toxicity mechanisms that prevent till now the use of a "TEQ-like" approach [11].

Data on the occurrence of NDL-PCB in food and feed have been reported in different ways as the sum of six PCB congeners (PCB 28, 52, 101, 138, 153, 180) often referred to as indicator PCB or as the sum of seven (sum of six indicator PCB plus PCB 118), because these congeners are appropriate indicators for different PCB patterns in various sample matrices and are most suitable for a exposure assessment of NDL-PCB on the basis of the available data. The sum of the six indicator PCB represents about 50% of total NDL-PCB in food [12].

Farm animals exposure

Feed is, by far, the major way of PCB intake by food-producing animals. Congener patterns in feed, particularly that of plant origin, may differ considerably from those in tissue residues. Due to the different sources of contamination, different origins of the feed and of food commodities, there is generally no correlation between the concentrations of NDL-PCB and DL-PCB.

Following exposure of farm animals, PCB will accumulate in meat, liver and particularly in fat tissues. In addition, PCB will be transferred into milk and eggs, and levels in these products will reach a steady state following exposure over a period of several weeks [13]. PCB 138 and 153, both with six chlorine atoms, show the highest carry-over into milk and eggs, in the order of 50-60% [14]. After cessation of exposure, levels in eggs and milk initially decrease rapidly to about 50%, followed by a slower elimination phase. In fattened animals like calves, piglets, and poultry, and also farmed fish, no steady state is obtained, since these animals are slaughtered at a young age.

For risk assessment of domestic animals, the EFSA Panel compared the effect concentrations in experimental diets with the NDL-PCB concentration in animal feed. Following a conservative approach, the levels of the sum of six NDL-PCB in compound feed, would be 0.02 mg/kg feed, corresponding to about 0.04 mg total NDL-PCB, which is more than two orders of magnitude below the concentrations causing effects in most domestic animals studied [12].

Two major situations of contamination of animal feeds, hence of foods of animal origin, may be identied according to the available literature. One is aquaculture, due to the general use of fats and proteins of animal origin (aquatic organisms) in fish feeds, that are therefore vulnerable to the contamination by cumulative xenobiotics; nevertheless, the EFSA has found no major differences as regards the content of PCB and PCDD/PCDF between farmed and wild fish [15].

The other situation is the pollution of pastures by improper disposal of chemical waste. In this case long-term intake by dairy animals may occur, leading to a significant excretion in milk; indeed, daity animals have productive lifes much longer than meat-producing animals. Effects in farm animals might act as sentinels of PCB contamination. However, animals may apparently tolerate long-term exposures, with no or only subtle effects [12].

A recent episode of PCB contaminating pastures has occurred in the industrial municipality of Brescia (Northern Italy) and is discussed below in detail.

Pasture contamination: the Brescia episode

A chemical factory located in Brescia, a highly industrialized town in Northern Italy, produced PCB for about 50 years. Those compounds polluted water which was discharged in irrigation channels and thence accumulated in the soil of a nearby agricultural area and entered the food chain.

Three different zones were present in the area around the industrial plant: an agricultural zone, a built-up urban zone, and a mixed zone such as public park and private gardens.

High microcontaminants levels in soils were observed: total levels of 57 NDL-PCB congeners varied from 81 ng/g to 18700 ng/g, exceeding the legal limit of 0.001 mg/kg [16]; DL-PCB levels from 7 to 400 pgWHO-TE/g, PCDDs and PCDFs from 8 to 592 pgWHO-TE/g; also these levels were higher than the limits for public and private gardens (10 pgTE(I)/g) [16].

The analytical results are detailed elsewhere [17, 18].

Surface water samples taken in the Mella river, west of the industrial plant, and in irrigation channels showed a different sharing out of contamination in relationship to industrial plant: the PCB levels were lowest than 0.01 µg/l at North, but varied from 0.02 µg/l to 0.18 µg/l in the South [17].

The most polluted area, situated South of the PCB producing factory, includes some farms, producing feedingstuffs and food of animal origine.

The total PCB levels in forage samples, varied from 20 to 700 ng/g dry forage and indicate a diffuse and significant contamination [17, 18]. The PCDD/F levels varied from 0.29 to 2.04 pg TE(WHO)/g forage [18], over the legal limit of 0.75 pg WHO TE/g, requiring the elimination of the feed and over the action level, because of presence of significant contamination demand investigation about the contamination source.

PCB levels in animal food (beef, poultry, eggs, milk), measured by different labs expressed as the sum of 7 indicator or 18 PCB congeners, [17, 19, 20], exceeded the action levels of 100 ng/g fat indicated in the Italian National Plan for Residues and Contaminants in food of animal origin (NRP) [21].

Also the TEQ levels for PCDD/F and DL-PCB measured in food were 10-fold higher than the EU limits of 3 pgTE/g of lipid for beef meat and for milk [19].

Most of vegetable samples, including radicchio, courgettes, onions, carrots, show PCB levels lower than 4 ng/g, except for some samples with PCB concentration 3-fold higher [17].

For beef, poultry, eggs and milk are measured the range values of 7 indicator PCB (28, 52, 101, 118, 138, 153, 180) considering the levels found in the samples [17] *(Table 1)*.

Considering the average level of PCB in these food, transformed on the fresh weitgh basis, and the Italian food intake [22], it is possible to calculate the daily intake of farmers consuming these products. The result of 140 ng/kg bw (assuming 60 kg bw) is 4-fold higher than the EFSA estimated intake for fish consumers.

Consequently the PCB levels in blood farmers were very high [17, 20, 23].

Human exposure

More than 90% of human exposure derives from food. By far, the major portion is due to the presence of residues in foods of animal origin with the contribution of each commodity being roughly related to the lipid content.

The final report of Scientific Cooperation (EU SCOOP) Task 3.2.5 provide information on dietary intake of PCDD/F and DL-PCB by population of EU Member States and on concentrations of these microcontaminants in food products and. The main contributors to the average daily dietary exposure to dioxins appear to be milk and diary products (16-39%), meat and meat products (6-32%), fish and fish products (11-63%); other products such as vegetables, cereals and fruit contribute some 6-26% [24].

The Scientific Committee on Food estimates the average human intake of PCDDs, PCDFs and DL-PCB from 1.2 to 3.0 pg WHO TEQ/kg bw/day in Europe [7, 25].

The EFSA Scientific Panel on Contaminants estimates the average daily dietary intake of NDL-PCB around 15 ng/kg bw (assuming 60 kg bw) per day for the "average" consumer, 20 ng/kg bw per day for the high consumer of meat and meat products and around 35 ng/kg bw per day for the high consumer of fish and fish products. Those results are consistent with the various assessments performed using more accurate national data [12].

Limited exposure data for young children, up to six years of age, indicates that the average intake (breastfeeding excluded) of total NDL-PCB is about 27-50 ng/kg bw per day. However, where data on both adults and children within a specific population were available, in general children had exposure levels 2.5 fold higher than adults.

Breastfeeding is a significant source of intake for fat-soluble POPs in a specific lifestage. Breastfed infants are a group of high NDL-PCB intake which might be two orders of magnitude higher than adult exposure [11]. The concentration of POPs in breast is greater in older, primiparous mothers and is in close relation with the maternal diet, with higher fish consumption [26].

In specific subpopulations with high dietary PCB exposure, such as fishermen of the polluted Baltic Sea area, the daily intake from fish of the sum of the six NDL-PCB could be about 40 ng/kg bw, corresponding to an intake of total NDL-PCB of 80 ng/kg bw per day before taking into account the rest of the diet [27].

Table 1 | *Range of seven indicator PCB levels measured in food matrices sampled in Brescia municipality (from Azienda Sanitaria Locale Brescia, 2003. Modified from [17])*

Food matrices	Samples unit	7 PCB* (min-max levels; ng/g fat)
Bovin fat	28	165 -1354
Rabbit	3	143-274
Poultry	5	64-6700
Eggs	16	85-13000
Milk	15	< 20-500

PCB: polychlorinated biphenyls.
**28,52,101,118,138,153,180.*

TOXICITY

Technical PCB mixtures used in toxicity studies contain both NDL-PCB and DL-PCB. These mixtures as well as individual congeners exert a variety of toxicological effects on a multiple systems such as liver, thyroid, immune function, reproduction and neurological development as well as carcinogenicity.

The most relevant aspect of PCB risk assessment is the evaluation of the long-term effects to low levels including carcinogenicity and endocrine disruption [28].

Due to the high number of congeners a functional grouping of PCB has been proposed as a pragmatic tool for toxicological risk assessment:

- group I (estrogenic: 44,49,52,101,174,177,187,201),
- group II (the DL-PCB, PCB 126, anti-estrogenic, immunotoxic, CYP1A enzime-inducing),
- group III (very persistent in the body, CYP1A1 and CYP2B enzime-inducing (phenobarbital like) (99, 153,180,183,196,203) [29, 30].

The International Agency for Research on Cancer (IARC) classified PCB as probably carcinogenic to humans (Group 2A), based on limited evidence in humans and sufficient evidence in animals [31]. Evaluation of the cancer studies in rats with technical PCB mixtures, and comparison with data obtained with TCDD, indicate that the DL components in technical PCB mixtures are likely to be responsible for the carcinogenic effect. No peer-reviewed data are available on the carcinogenicity of individual NDL-PCB congeners. They may act tumor promoters in tissues such as liver. The mechanisms of their promoting activities may include the induction of oxidative stress and inibition of apoptosis with different way related to different congeners [32]. Several epidemiological studies attempted to associate biomarkers of PCB exposure with different kinds of cancer, especially breast cancer, but results were inconsistent [33]. However, in evaluating the tumorigenicity of PCB in human populations, consideration has to be given to susceptibility factors associated to genetic polymorphisms of biotransformation genes. A cohort study on breast cancer and PCB observed a significant elevated risk of postmenopausal breast cancer associated with elevated serum PCB levels and the presence of specific polymorphism of CYP1A1 enzyme [34].

Early predisposition of tissue to cancer onset later in early ages may also deserve more attention; the perinatal exposure of rats to PCB 126 impaired the histological differentiation of mammary glands in rats, leading to changes possibly related to enhanced cancer risk [35].

The overall evidence points out that a) PCB carcinogenicity is a secondary consequence to other, complex effects; b) the effects of PCB on endocrine nervous and immune systems are the critical parameters for the toxicological risk assessment of both DL and NDL-PCB, and c) the developing lifestages (uther, prenatal and peri-postnatal) are the most vulnerable to PCB actions.

NDL- and DL-PCB have to be both considered as endocrine disrupters, potentially being able to alter a number of hormone systems such as thyroid and sexual hormones [36]. Although mechanisms are still to be completely clarified, recent data indicate that thyroid is an important target for PCB 126, that may disrupt both the regulation and the metabolism of thyroid hormones [37].

Several NDL-PCB congeners are metabolised to hydroxy-PCB and/or methylsulfonyl-PCB. Some of these metabolites may contribute to hormone-like effects seen with PCB. In particular, the hydroxy-PCB may inhibit sulfotransferases, thus increasing the amount of free estrogens available to the organism [38].

A possible critical aspect for risk assessment of "background" intakes of PCB is the induction of subtle neurobehavioral alterations in children [39, 40]. The subtle effects associated with PCB exposure in human studies are consistent with the neurodevelopmental effects observed in the offspring of rodents following in utero exposure [41]. Such effects are shatred by NDL-PCB, DL-PCB and PCDD/PCDF.

Some PCB effects in humans are been directly related to food of animal origin. For instance, in the highly PCB-polluted area of the American Great Lakes, increased consumption of contaminated fish has been associated with lower markers of thyroid function [42-44], reduced mean birthweight [45] and altered sex-ratio with an increased proportion of males [46].

Overall a number of experimental and epidemiological studies consistently indicate that reproductive and children health are the critical targets for risk assessment of both DL-PCB and NDL-PCB [47,12].

CONSIDERATIONS ON RISK ASSESSMENT AND MANAGEMENT

The Council Regulation of the European Community [48] fixed maximum levels for PCDD/F and DL-PCB in foodstuffs of animal origin, to prevent exposure of the human population *(Table 2)*. DG SANCO proposes the maximum levels for PCDD/F and DL-PCB in feedstuffs reported in *(Table 3)* [49].

Recently the European Commission indicated the action levels in foodstuffs for PCDD/F and DL-PCB, so that the Member States have to individuate the cases where significant levels are found above the normal background levels and identify a source of contamination [50].

For these compounds the Scientific Committee on Food has established in 2001 a Tolerable Weekly Intake (TWI) of 14 pg WHO TEQ/kg bw, that is at least 5-fold higher than the average background intake in Europe [25]; however, the recent EFSA Scientific Colloquium has endorsed a possible revision according to the most recent findings of toxicological research [12].

It is remarkable that no maximum levels has been established for NDL-PCB, which are the major component of PCB in feeds and foods.

The Italian National Plan for Residues and Contaminants in food of animal origin (NRP), indicated the action levels of 100 ng/g fat for 18 PCB congeners (28, 52, 95, 99, 101, 105, 118, 138(+163), 146, 149, 153, 170, 177, 180, 187) in food, except for fish because of their high background values [21]; Italy is one of the few EU Countries with official action limits for some NDL-PCB congeners in food [24].

Table 2 | *Maximum levels sum of PCDD/F and DL-PCB in foodstuffs of animal origin fixed by the Council Regulation of the European Community (2006/199/EC)*

Products	Maximum levels (pg WHO-PCDD/F-PCB TEQ/g fat)
Meat and meat products originating from:	
ruminants (bovine animals, sheep)	4.5
poultry and farmed game	4
pigs	1.5
Liver and derived products	12
Muscle meat of fish and fishery products and products thereof	8*
Milk and milk products, including butter fat	6
Hen eggs and egg products	6
Oils and fats:	
animal fat	
of ruminants	4.5
of poultry and farmed game	4.0
of pigs	1.5
mixed animal fat	3
vegetable oil	1.5
Fish oil intended for human consumption	10

PCDD/F, DL-PCB: polychlorodibenzodioxins, polychlorodibenzofurans and dioxin-like polychlorinated biphenyls.
** pgWHO-PCDD/F-TEQ/g fresh weight.*

The NDL-PCB in feeds and foods were considered recently by EFSA; the Scientific Panel on Contaminants concluded that no health based guidance value for humans can be established for NDL-PCB because simultaneous exposure to NDL-PCB and dioxin-like compounds hampers the interpretation of the results of the toxicological and epidemiological studies, and the database on effects of individual NDL-PCB congeners is rather limited [12].

There are however indications that subtle developmental effects may occur at maternal body burdens only slightly higher than those expected from the average daily intake in European countries. Because some individuals and some European (sub)-populations may be exposed to considerably higher average intakes, a continued effort to lower the levels of NDL-PCB in food is warranted [12].

Table 3 | *Maximum content of PCDD/F and DL-PCB relative to a feedingstuff with a moisture content of 12% (as proposed by SANCO/00362/2005)*

Products intended for animal feel	Maximum content (ng WHO-PCDD/F-PCB-TEQ/kg)
Feed materials of plant origin with the exception of vegetable oils and their byproducts	1.25
Vegetable oils and their by-products	1.5
Feed materials of mineral origin	1.5
Animal fat, including milk fat and egg fat	3
Other land animal products including milk and milk products and eggs and egg products	1.25
Fish oil	24
Fish, other aquatic animals, their products and by-products with the exception of fish oil and fish protein hydrolysates containing more than 20% fat	4.5
Fish protein hydrolysates containing more than 20% fat	11
Additives	1.5
Premixtures	1.5
Compound feedingstuffs, with the exception of feedingstuffs for animals, pet foods and feedingstuffs for fish	1.5
Feedinstuffs for fish and pet foods	7

PCDD/F: polychlorodibenzodioxins and polychlorodibenzofurans.
DL-PCB: dioxin-like polychlorinated biphenyls.

PCB may be viewed as a widespread, low-intensity problem. Nevertheless situations of comparatively high exposure may lead to questions about whether, farming practices or dietary habits should change.

Two examples of such instances are the consumption of fish and the practice of breast-feeding.

As for the consumption of wild and farmed fish the EFSA recommends that the current levels of contaminants, including PCB, would not justify a general recommendation against the consumption of fish, that has in fact some significant dietary advantages (*e.g.*, intake of omega-3 fatty acids, iodine, etc.). Nevertheless, at EU level resources should continue to be devoted to PCB monitoring; specific risk management and risk communication actions may be needed in highly polluted areas, such as the Baltic Sea. EFSA recommends also that research should be carried out on novel and "cleaner" feeds for fish farming [15].

Breast-fed infants have higher intakes of fat-soluble pollutants (including PCB) for a short but developmentally important part of their lives [26]. However, WHO recommends to promote and support breast-feeding since the numerous benefits (health, nutritional, immunologic, developmental, psychological and social) are widely recognized to outweigh the risks at the background contamination levels. On the other hand, efforts should be made to control and reduce the levels of contaminants in the diet of pregnant and lactating women as well as in infant foods [51].

In general, PCB effects on animals and humans are subtle but their widespread diffusion make them important at population level. Reports on slowly increasing levels in foods and organisms may be reassuring, on the other hand an additive effect with other emerging persistent chemicals, such polybrominated flame retardants cannot be ruled out completely. It is likely that in the next years PCB will remain an issue for research in veterinary public health and food safety.

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