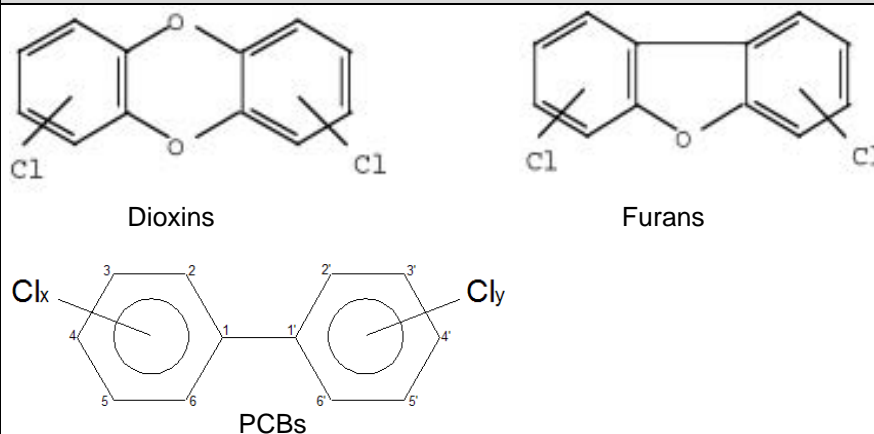


DIOXINS AND DL PCB

The term "dioxin" is used to refer to polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofurans (PCDF). There are in total 75 congeners of PCDD and 135 congeners of PCDF. Seven PCDDs and 10 PCDFs with a substitute in position 2, 3, 7 and 8 are considered toxic. The most toxic compound is the 2, 3, 7, 8-Tetrachlorodibenzodioxin (TCDD) or "Seveso dioxin".

Polychlorinated biphenyls (PCBs) are a group of organochlorine compounds that are synthesized by catalyzed chlorination of biphenyl. Depending on the number of chlorine atoms (1-10) and their position on the two rings, 209 different compounds, also named "congeners" are possible. Based on structural characteristics and toxicological effects, PCBs can be divided into two groups. One group consists of 12 congeners that easily can adopt a coplanar structure and show toxicological properties similar to dioxins. This group is therefore called "dioxin-like PCBs" (DL-PCBs). The other PCBs do not show dioxin-like toxicity and have a different toxicological profile. This group is called "non dioxin-like PCBs" (NDL-PCBs) (See also fiche about PCB).

Chemical structure



PCDDs are consisted of two benzene rings connected by two atoms of oxygen while PCDFs differ from PCDDs by the presence of a single atom of oxygen in the central cycle. The chlorine content of these molecules can vary from 1 to 8 atoms.

PCBs are characterized by two benzene rings attached by a carbon-carbon link. Their general formula is $C_{12}H_{(10-n)}Cl_n$.

Contamination source

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are by-products of combustion and of various industrial processes, and they are widely present in the environment (JECFA, 2002).

There are two ways of synthesis of dioxins and furans. On one hand, **synthesis de novo**, resulting from the secondary reaction between carbon and oxygen in the presence of chlorine and a catalyst at temperatures of about 350°C. This route of synthesis focuses on the process of incineration of household waste, hospital waste and all processes involving a step of combustion, such as production of iron and steel; incineration of domestic waste; combustion oil, diesel, wood heating, electricity, tobacco smoke or natural causes such as forest fires and volcanic eruptions. The *de novo* synthesis is currently the main way of producing dioxins and furans.

On the other hand, **the synthesis of precursors**. 2,4,5-trichlorophenol used in the synthesis of herbicide phenoxyacetic acid (2,4,5-T) may, by condensation reaction/cyclization, give birth to 2,3,7,8-TCDD. Similarly, pentachlorophenol, formerly used in the conservation of wood, can produce octa-CDD. Another example is the degradation of PCBs (Aroclor), as components of oil processors, into dibenzofurans, as a result of a phenomenon of pyrolysis (Eppe *et al.*, 2006).

Dioxins have no technological or other use, but are generated in a number of thermal and industrial processes as unwanted and often unavoidable impurities or by-products. Dioxins are generally not generated as single congeners but mostly as complex mixtures which are often characteristic of the source. Due to the numerous sources, dioxins are ubiquitous. However, due to a number of regulatory measures since the 1980s the emission of dioxins into the environment has considerably decreased (EFSA, 2011).

In contrast to dioxins, PCBs had widespread use in numerous industrial applications, generally in the form of complex technical mixtures. As a result of their widespread use, leakages and improper disposal practices, PCBs (like dioxins) also have a global distribution in the environment where they are persistent because they are poorly degraded and thus they are bioaccumulated in the food chain. Like dioxins, PCBs belong to the initial list of 12 persistent organic pollutants (POPs) that are regulated under the Stockholm Convention on POPs (EFSA, 2011).

Dioxins and dioxin-like PCBs are fat soluble and tend to bioaccumulate in body fat, both in animals and humans, biomagnifying through the food chain. They are generally not taken up or absorbed by plants, with the exception of some members of the cucurbit family, but may settle on the surfaces of the leaves. They can then enter the food chain when animals eat the contaminated leaves. In aquatic environments, fish and other marine animals can absorb dioxins and dioxin-like PCBs (EFSA, 2010).

Analytical method

PCDDs, PCDFs and dioxin-like PCBs are found at levels as low as pico- or femtogram per gram of matrix depending on the investigated food sample. In addition, matrix-related interferences are present in concentrations at orders of magnitude higher than the analytes of interest. For those reasons, a complex multi-step approach is required to (1) extract the analytes from the matrix core, (2) separate undesirable interferences and (3) finally isolate, separate and quantify analytes of interest (Scippo et al., 2008).

Different methods are used to determine the levels of PCDD/Fs and DL-PCBs (Baeyens et al., 2004; Scippo et al., 2008) in environmental as well as in food and feed matrices. A distinction is made between the so-called 'reference method' and the alternative or screening methods. Gas chromatography (GC) in combination with high resolution mass spectrometry (HRMS) is used as the reference method ('the golden standard') for the identification and quantification of PCDD/Fs and DL-PCBs (Baeyens et al., 2004; Behnisch et al., 2001; Firestone, 1991; Liem, 1999). For screening, bioassays can be used to determine a biological response towards PCDD/Fs and DL-PCBs such as enzyme activity, the expression of reporter genes, the binding between ligand and receptor or antigen and antibody. The cell based assay CALUX (Chemical-Activated LUciferase gene eXpression), a widely used screening method, is used successfully for feed and food.

The CALUX assay is a reporter-gene-cell based assay using genetically modified murine hepatoma cells which respond to chemicals able to activate the Ah Receptor by producing firefly luciferase (Denison et al., 2004; Han et al., 2004; Van Overmeire et al., 2001).

Detailed requirements for methods of sampling and analysis for the official control of levels of dioxins and DL-PCBs in certain foodstuffs are laid down in Commission Regulation (EC) No 1883/2006.

Toxicity

Dioxins and dioxin-like PCBs (polychlorinated biphenyls) form a group of toxic and environmentally persistent chemicals whose effects on human health include dermal toxicity, immunotoxicity reproductive effects and teratogenicity, endocrine disrupting effects and carcinogenicity (Van den Berg *et al.*, 1998).

Toxicity of dioxins and dioxin-like PCBs is mainly mediated through binding to the aryl hydrocarbon (Ah) receptor thereby inducing protein synthesis.

Various effects have been reported in animals exposed to PCDDs, PCDFs and PCBs. Many of the toxic effects of dioxins were high dose effects (SCF, 2001; JECFA, 2002). The most commonly reported pathologies are endometriosis, immunotoxic effects, cancer, birth defects, effects on the reproductive and the neuro-endocrine, immune systems, altered metabolism and specific organ dysfunction (EPA, 2006).

TCDD biological half-life in human is 7 to 10 years.

Dioxins are reputed to be among the most toxic of organic compounds.

Carcinogenicity

Chronic exposure of animals to dioxins has resulted in several types of cancer. Based on both animal studies and epidemiologic evidence, 2,3,7,8-TCDD was classified as a "known human carcinogen" (class 1) by the International Agency for Research on Cancer (IARC) in 1997. Other PCDDs and PCDFs were considered not to be classifiable as their carcinogenicity to humans (group 3) (JECFA, 2002).

2,3,7,8-TCDD does not directly affect genetic material and there is a level of exposure below which cancer risk would be negligible.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is carcinogenic in experimental animals, but has not been conclusively proven to cause cancer in humans. Indeed, evidence for an effect in humans has remained controversial (Boffetta et al., 2011). Bertazzi et al. (2001) observations have showed an increase of rectal and lung cancer cases, 20 years after the 1976 Seveso accident. However, there is no consensus in the scientific community on the carcinogenicity of TCDD, controversial for some (Cole et al., 2003) and confirmed for others (Steenland et al., 2004).

The overall evaluation leading to classification in IARC Group 1 (established human carcinogen) was based on mechanistic considerations on the role of the aryl hydrocarbon (Ah) receptor in TCDD-related carcinogenesis in both humans and animals. The same mechanism was used to justify emphasis on the risk of all cancers combined rather than specific cancer sites (Boffetta et al., 2011). In 2009 IARC reviewed the carcinogenicity of TCDD as part of a systematic reassessment of all agents classified in Group 1 and classified the evidence of carcinogenicity in humans as sufficient, based on increased risk of all cancers combined (Baan et al., 2009).

In the IARC evaluations evidence of a causal association with TCDD exposure was considered strongest for lung cancer, non- Hodgkin lymphoma (NHL), and soft-tissue sarcoma (STS) (Baan et al., 2009).

Dioxins and among those especially TCDD, are so called multiple-site, multiple-species carcinogens. This means that they cause cancer in different animal species in various organs.

Dioxins are not DNA-reactive, i.e., they do not bind covalently to nucleic acids. That is why other mechanisms have been proposed by which these substances cause tumours. A large number of studies have been conducted, most of them with the model compound TCDD (EFSA, 2011).

Life-long exposure to TCDD resulted in a pronounced increase in the incidence of hepatocellular carcinoma in female rats, whereas the carcinogenic potency of TCDD in the liver was much less pronounced in males. Furthermore, TCDD caused an increase in tumour incidence in a number of different tissues in both sexes, e.g., tongue, nasal turbinates, hard palate and lung. In addition, male rats developed thyroid tumours. Interestingly, TCDD caused a decrease in the occurrence of some oestrogen-dependent tumours, e.g. in the uterus and mammary gland. It was discussed that an anti-estrogenic effect of TCDD might explain this finding (EFSA, 2011).

Endocrine effect

In laboratory animals, TCDD can directly affect reproductive organs in both males and females, on the other hand TCDD interferes with steroid hormone homeostasis. In particular, TCDD acts on estradiol homeostasis. This effect is thought to contribute to the anti-estrogenic actions of dioxins. Exposure of adult male rodents to TCDD decreases serum androgen concentrations and affects male fertility. In adult rats TCDD also causes a decrease in T4 and concomitant increase in thyroid stimulating hormone (TSH) (EFSA, 2011).

Effects of dioxin-like substances on sexual dimorphic behaviour, brain morphology and sex ratios in offspring and the link between dioxin body burden and altered menstrual cycle characteristics observed in the Seveso cohort as described by Eskenazi *et al.* (2002) point to dioxin-like compounds playing a role as endocrine disrupters in the system of sex steroidal hormones. The role of dioxin-like substances in disruption of the thyroid hormone system has been extensively reviewed (IPCS, 2002).

Endocrine disrupters in general and dioxin in particular can exert non monotonic dose-response curves and can show different effects in different windows of critical exposure (German Federal Environmental Agency, 2002).

Others effects

TCDD is known to cause chloracne. Chloracne represents a hyperkeratotic skin disorder, which affects the hair follicles, sebaceous glands and interfollicular epidermis. It is the most consistently observed pathology in exposed humans since 1957 when dioxins were first identified as culprits for chloracne (Bock & Köhle, 2006).

TCDD could be one of the multiple factors causing atherosclerosis, hypertension, vascular ocular changes and signs of neural system damage.

The concept of toxic equivalency

For risk assessment purposes, the concept of toxic equivalency (TEQ) was developed to describe the cumulative toxicity of complex mixtures of these compounds (Ahlborg *et al.*, 1992). The procedure involves assigning individual toxicity equivalency factors (TEFs) to the PCDD, PCDF, and PCB congeners in terms of their relative toxicity compared to 2,3,7,8-TCDD, which is considered as the reference congener (TEF=1). The toxic equivalency (TEQ) of a mixture is calculated by multiplying the concentrations of individual congeners by their respective TEF, and then adding the individual TEQs to obtain a total TEQ concentration for the mixture (EFSA, 2010).

Table 1 Toxicity equivalency factors (TEFs) proposed by WHO in 1998 and in 2005. Changes are in bold.

Compound	TEF _{WHO98}	TEF _{WHO05}	Compound	TEF _{WHO98}	TEF _{WHO05}
Chlorinated dibenzo-p-dioxins			Non-ortho substituted PCBs		
2,3,7,8-TCDD	1	1	PCB-77	0.0001	0.0001
1,2,3,7,8-PeCDD	1	1	PCB-81	0.0001	0.0003
1,2,3,4,7,8-HxCDD	0.1	0.1	PCB-126	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1	PCB-169	0.01	0.03
1,2,3,7,8,9-HxCDD	0.1	0.1			
1,2,3,4,6,7,8-HpCDD	0.01	0.01			
OCDD	0.0001	0.0003			
Chlorinated dibenzofurans			Mono-ortho substituted PCBs		
2,3,7,8-TCDF	0.1	0.1	PCB-105	0.0001	0.00003
1,2,3,7,8-PeCDF	0.05	0.03	PCB-114	0.0005	0.00003
2,3,4,7,8-PeCDF	0.5	0.3	PCB-118	0.0001	0.00003
1,2,3,4,7,8-HxCDF	0.1	0.1	PCB-123	0.0001	0.00003
1,2,3,6,7,8-HxCDF	0.1	0.1	PCB-156	0.0005	0.00003
1,2,3,7,8,9-HxCDF	0.1	0.1	PCB-157	0.0005	0.00003
2,3,4,6,7,8-HxCDF	0.1	0.1	PCB-167	0.00001	0.00003
1,2,3,4,6,7,8-HpCDF	0.01	0.01	PCB-189	0.0001	0.00003
1,2,3,4,7,8,9-HpCDF	0.01	0.01			
OCDF	0.0001	0.0003			

Establishment of Health Based Reference Values

On basis of the LOAEL of 25 ng/kg bw for decreased sperm production and altered sexual behavior in mal offspring determined by Faqi *et al.* (1998) the SCF (2001) has established a TWI of 14 pg/kg /week

Occurrence in food

Assessments of exposure by the European Commission (2000) and the EPA (2000) in the USA showed that > 90% of the exposure of a typical person to PCDDs, PCDFs and coplanar PCBs came from food and predominantly from animal fat.

PCDD/F as well as DL-PCBs can be absorbed through animal products (milk, butter, meat and fat) and seafood (fatty fish, crustaceans, molluscs). Once absorbed, PCDD/Fs and DL-PCBs accumulate mainly in the lipophilic proteins of blood, liver and fat tissue.

A common hypothesis explaining the presence of dioxins in livestock is that animals consume food contaminated by emissions from combustion sources via atmospheric depositions.

Meat from eels and fish liver and derived products contain the highest average contamination levels of both dioxins and PCBs in Europe (EFSA, 2012).

Concentration of PCDD/Fs and DL PCB measured in a Belgian pooled sample of human milk collected in 2006 during the fourth World Health Organization Human biomonitoring campaigns was

17.33 ng/g lipid (Colles et al., 2008). The PCDD/Fs concentration in Belgian human milk follow the international downward tendency. In this fourth survey Belgian levels decreased again down to 10.3 pg WHO1998-TEQ/g lipid base. This is comparable to concentrations detected in the third survey five years ago in Finland, Sweden, the Czech Republic, Romania, Slovakia, Spain and Italy (between 7.8 and 12.7 pg TEQ/g lipid base).

Dietary exposure assessment

The mean dietary intake of PCDD/Fs and DL-PCBs in the Belgian adult population in 2008 was estimated to be 0.72 pg TEQ /kg body weight (bw)/day (middle bound concentrations, TEF of 1998) based on occurrence data of 2008 and national food consumption data of 2004 (Windal et al., 2010). When using the 2005 TEF instead of the 1998 TEF, the mean dietary intake in the Belgian adult population was estimated to be 0.61 pg TEQ /kg bw/day. The 95th percentile dietary intake of PCDD/Fs and DL-PCBs in the Belgian adult population was estimated at 1.37 pg TEQ/kg bw/day.

The average exposure among adults to dioxins and DL PCBs is estimated, in France, to 53.7 pg TEQ/kg bw/month, or 1.8 pg TEQ/kg bw/day (median 1.5 pg TEQ/kg bw/day) and among children, to 82.7 pg TEQ/kg bw/month, or 2.8 pg TEQ/kg bw/day (median 2.4 pg TEQ/kg bw/day). This more important level for children (3-14 years) is to be commensurate with the level of food consumption in larger proportion to the body weight (AFSSA, 2006). Several European countries (Finland, Netherlands, United Kingdom) published in 2004 exposure estimations of their population to PCDD/Fs and DL-PCBs. The average levels for adults from these countries are 3.2 pg TEQ/kg bw/day in Spain, 1.5 pg TEQ/kg bw/day in Finland, 0.9 pg TEQ/kg bw/day in the UK and a median level of 1.2 pg TEQ/kg bw/day in the Netherlands. Because of the use of different methodologies in these countries, comparisons are difficult. However, the estimated average exposure of the French population of 1.8 pg TEQ/kg bw/day for adults (median 1.5 pg TEQ/kg bw/day) is of the same magnitude as those these four countries (AFSSA, 2006).

Upper bound mean PCDD/Fs and DL-PCBs dietary exposure estimated recently by EFSA (2012) for the Belgian population are 1.77 pg WHO 05 TEQ/kg bw/day for toddlers in Flanders, 1.52 pg WHO 05 TEQ/kg bw/day for other children in Flanders, 0.65 pg WHO 05 TEQ/kg bw/day for adolescent, 0.82 pg WHO 05 TEQ/kg bw/day for adults, 0.99 pg WHO 05 TEQ/kg bw/day for elderly and 0.95 pg WHO 05 TEQ/kg bw/day for very elderly.

The major contributor to total exposure in Europe reported by EFSA (2012) was the food category of milk and dairy products for almost all groups of infants and toddlers, whereas it was fish and seafood for most of the groups of adolescent, adult, elderly and very elderly. Meat and meat products also contributed significantly to total exposure.

The background body burden of the Belgian population is estimated at 4.95 ng TEQ /kg bw (median value) for an adult of 50 years (Vrijens et al., 2002).

Data from various countries show that human exposure to dioxin and dioxin-like PCBs has declined in recent years. A general decrease in exposure to the sum of dioxins and DL-PCBs of between 16.6% and 79.3% across the different population groups in Europe was observed when comparing 2002-2004 data with data from 2008-2010 (EFSA, 2012). However, incidents such as the Belgian crisis of 1999 (Van Larebeke 2001, Bernard *et al.*, 2002) and others (Malish 2000, Llerena *et al.*, 2001) show that dioxin may pose a threat in terms of accidental contamination of the food chain.

Risk characterization

The Belgian dietary exposure of 0.72 pg TEQ/kg bw/day (or 5.04 pg TEQ/kg bw/week or 21.6 pg TEQ/kg bw/month) is clearly below the Tolerable Weekly Intake (TWI) of 14 pg TEQ/kg bw/week set by the Scientific Committee on Food of the European Commission and below the provisional tolerable monthly intake of 70 pg TEQ/kg bw/month set by the Joint FAO/WHO Expert Committee on Food Additives. Considering the cumulative distribution, the intake was less than 1 pg TEQ/kg bw/day for more than 80% of the population, and less than 2 pg TEQ/kg bw/day for the entire population (Windal et al., 2010).

Average exposure to dioxins and DL-PCBs was estimated to be between 0.57 and 2.54 pg WHO 05 TEQ/kg bw/day and the 95th percentile between 1.2 and 9.9 pg WHO 05 TEQ/kg bw/day in Europe. The percentage of individuals exposed above the Tolerable Weekly Intake (TWI) of 14 pg TEQ/kg

bw was estimated to be between 1.0 and 52.9 % (EFSA, 2012).

Legislation

Commission Directive 2002/32/EC of 7 May 2002 on undesirable substances in animal feed. *Official Journal of the European Communities* L 140, 10–22.

Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Official Journal of the European Communities* L 54, 26.2.2009, p. 1–130.

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, L364, 5-24.

Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin like PCBs in certain foodstuffs. *Official Journal of the European Union*, L84, 23.3.2012, 1-22.

Commission Recommendation 2011/516/EU of 23 August 2011 on the reduction of the presence of dioxins, furans and PCBs in feed and food. *Official Journal of the European Union* L 218, 23-25.

Recommendations

Recommendations of EFSA (2012)

- In order to improve the accuracy of the assessment of food contamination levels and exposure to dioxins and PCBs throughout Europe, it is important to clearly define the sampling strategy used both at the sample level and for the overall direction of monitoring programs. Further, results should be reported with a clear indication of the unit of expression of the result (on fat, whole weight or moisture basis), as it greatly impacts the estimation of the contamination levels of food and feed to dioxins and PCBs.
- It is recommended to measure dioxins and DL-PCBs in those foods identified as main contributor to the total exposure of the population, but for which the estimations of the contamination levels were not robust

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