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Brominated dioxins: little-known new health hazards - a review

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Abstract

This article reviews the present state of the science concerning the polybrominated dibenzo-p-dioxins (PBDDs) and dibenzofurans (PBDFs). Everywhere in the world people are exposed to anthropogenic origin chemicals. Some of them are long-lived organic compounds, which persist over the years in the environment. Persistent organic pollutants, such as organohalogen compounds, accumulate in environmental and biological compartments and have adverse effects on the health of humans and animals. Little is known about the brominated and mixed chloro/bromo dioxin and furans. Existing literature suggests that brominated dioxins and furans have similar toxicity profiles to their chlorinated analogues. The exposure data are extremely limited, showing a major data gap in estimating the potential environmental and health risk of these chemicals. The rapid increase in the use of brominated flame retardants (the main source of these pollutants) has raised the level of concern over environmental and health damage from brominated dioxins and furans. It is likely that human as well as wildlife exposure to these contaminants will increase with their greater use. The findings reported here present strong evidence of the PBDDs and PBDFs as an emerging new class of contaminants.

Keywords: brominated dioxins, toxicity, occurrence, risk.

Introduction

Everywhere in the world people are exposed to anthropogenic origin chemicals. Some of them are long-lived organic compounds, which persist over the years in the environment. Persistent organic pollutants, such as organohalogen compounds, accumulate in environmental and biological compartments and have adverse effects on the health of humans and animals. The international community has called for urgent global action to reduce and eventually eliminate the release of POPs to the environment. Among POPs covered by the Stockholm Convention are polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The chlorinated dioxins and dioxin-like compounds (PCDDs, PCDFs, and dioxin-like polychlorinated biphenyls (DL-PCBs)) are environmental burdens subject to reduction measures. All organohalogen compounds on the initial POP list are chlorinated. In contrast to chlorinated dioxins, knowledge on brominated dioxins is very limited.

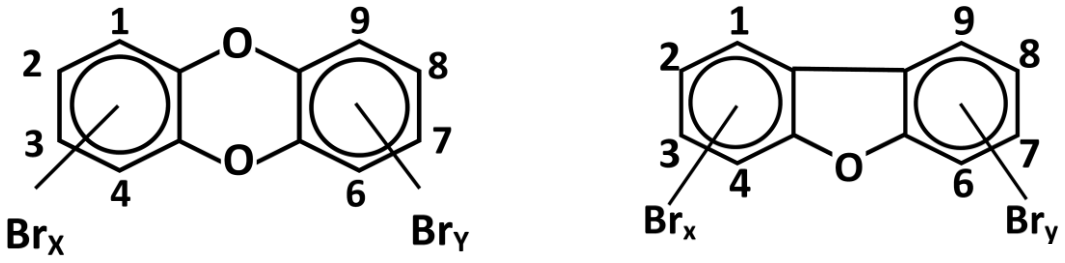
Advances in analytical chemistry during the last decade and new toxicological studies show that in addition to the 29 toxic chlorinated dioxin and dioxin like congeners already well-known and covered by European regulation 1259/2011/EU, there is a very large group of brominated and mixed chloro/bromo dioxin and furan analogues (9, 11, 37). Relatively little is known about the environmental and toxicological significance of polybrominated dibenzo-p-dioxins (PBDDs) and polybrominated dibenzofurans (PBDFs). While chlorinated dioxins have been studied intensively, brominated and mixed bromo/chloro analogues have been studied to a much smaller extent. Existing literature suggests that brominated dioxins and furans (PBDDs/Fs) have similar occurrence profiles to their chlorinated analogues, but the data is extremely limited, showing a major gap in estimating the potential risk of these chemicals. This is caused by the relatively low recent global recognition of their environmental distribution and toxicological significance, also the difficulties in analytical accessibility in order to measure them.

Why we should devote more attention to this group of brominated compounds? The growing problem arises from several reasons: the similarities in the chemical structure of brominated and chlorinated dioxins and furans which determine their biological action; evidence for a mechanism of action common to both brominated and chlorinated analogues; the similar sources of formation of both brominated and chlorinated dioxins; the confirmed presence in the environment; the same evidence in animals; confirmed human contact with these compounds; and, above all, steadily increasing worldwide use of brominated flame retardants (BFR), which are by their nature contaminated with brominated dioxins. All these suggest that brominated dioxins and furans may constitute a toxicological issue. A brief overview of the current state of knowledge regarding the PBDDs/Fs based on the available literature is presented below.

Structure and physicochemical properties. PBDDs and PBDFs are almost planar tricyclic aromatic compounds. The compounds vary in the number of bromine atoms and the positions of halogenations. There are eight positions on both the dibenzo-p-dioxin and the dibenzofuran molecules where halogen substitution can occur. The positions are numbered as shown in Fig. 1. Theoretically, 75 PBDDs and 135 PBDFs are possible (Table). In comparison to their chlorinated relatives (PCDDs and PCDFs), brominated dioxins and furans have higher molecular weights, higher melting points, lower vapour pressures, and lower water solubility. They are generally soluble in fats, oils, and organic solvents. Although the PBDDs and PBDFs are more lipophilic and less water-soluble than the PCDDs and PCDFs, the brominated compounds appear to be less environmentally persistent, and more sensitive to UV degradation. There is little experimental data on the physical and chemical properties of PBDDs/PBDFs (37).

Formation and sources. PBDDs/PBDFs are not known to occur naturally and they are not intentionally produced, but are generated as undesired by-products in various processes: chemical, photochemical, or thermal reactions from precursors and by *de novo* synthesis. It is widely accepted that chlorinated dioxins arise from combustion processes such as incineration. Combustion of organics in the presence of both bromine and chlorine results in the formation of mixed halogenated dibenzo-p-dioxins and dibenzofurans (*i.e.*, brominated, brominated/chlorinated and chlorinated). Burning of products containing brominated compounds causes the emission of PBDDs and PBDFs. The presence of PBDD/Fs and/or mixed brominated/chlorinated dibenzo-p-dioxins and dibenzofurans (PXDD/PXDFs) has been reported in the fly ash and flue gas of municipal, hospital, and hazardous waste incinerators (7, 13, 33, 36, 37). In addition to the anthropogenic sources of brominated dioxins, several biogenic pathways have been proposed, *e.g*. formation *via* environmentally abundant 2, 4-di- and 2, 4, 6-tribromophenol. Tri- and tetra- brominated dioxins may also be produced through biogenic formation *via* precursor pollutants in the marine environment and may bioaccumulate in some marine species such as shellfish (10, 15, 22).

Nonetheless, brominated flame retardants (BFR) and their precursors constitute the main source of PBDD/Fs, because PBDD/Fs are impurities in the widely used brominated flame retardants (16, 27, 29). Brominated dioxins and furans have been found in brominated flame retardants such as polybrominated diphenyl ethers (PBDEs), decabromodiphenyl (decaBB or DBB), 1, 2-bis (tribromophenoxy) ethane, tetrabromobisphenol A (TBBPA), and others.



Dibenzo-p-dioxins x + y = 1 – 8 Dibenzofurans

Fig. 1. PBDD and PBDF structural formulas

Table. Number of possible PBDD and PCDF congeners

Compound	Substitution: Mono	Di	Tri	Tetra	Penta	Hexa	Hepta	Octa	Total
PBDD	2	10	14	22	14	10	2	1	75
PBDF	4	16	28	38	28	16	4	1	135
								Total	210

Thermal degradation, photochemical transformation, sunlight exposure, ultraviolet irradiation, and recycling of waste containing BFR can yield significant amounts of PBDD/Fs. The burning of products containing BFRs as well as thermolysis of BFR material is an important source of PBDD/F emissions. Brominated dioxins and furans are impurities in plastics containing BFRs and also in the thermal breakdown products of material containing BFRs. BFRs may also transform into PBDD/Fs under thermal stress during manufacture (36).

The widespread use of brominated flame-retardant products during the last two decades has increased bromine emission, caused when these products undergo thermal processes such as waste combustion and accidental fires (13, 21, 29). Brominated and mixed brominated/chlorinated dibenzo-p-dioxins and dibenzofurans are micro-pollutants of concern arising from such processes. Weber *et al.* (36) describe four categories of thermal processes in respect to their potential for PBDD/F and PXDD/PXDF generation: thermal stress, pyrolysis/gasification, insufficient combustion conditions, and controlled combustion conditions. Under thermal stress situations, as may occur in production or recycling processes, PBDD and PBDF precursors like polybrominated diphenylethers (PBDE) may potentially form PBDD/Fs *via* a simple elimination. Under insufficient combustion conditions as are present in, *e.g*. accidental fires and uncontrolled burning as well as gasification/pyrolysis processes, considerable amounts of PBDD/Fs can be formed from BFRs, most readily *via* the precursor pathway (36).

Environmental levels of PBDD/Fs start increasing in step with rising production, use and disposal of brominated flame retardants, and recycling processes of electronic waste (Fig. 2). Plastic manufacturers produce BFR-containing fireproof plastics, and these release their constituent PBDD/Fs at several recycling stages. Electronic waste recycling with uncontrolled combustion processes is one of the major sources of PBDD/Fs (1, 16, 31, 33).

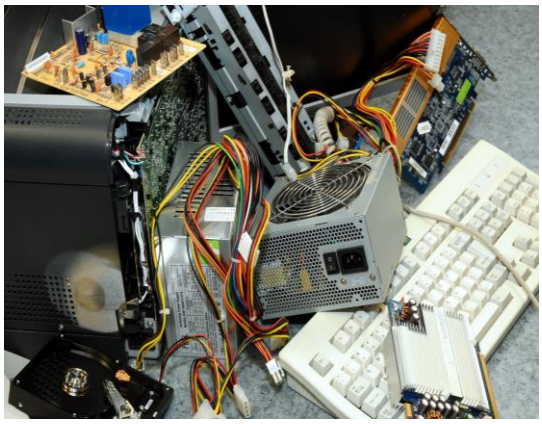


Fig. 2. Electronic waste

Modern buildings contain flame retardants in the plastic housings of electronics such as computers, televisions, copiers, and printers, as well as in upholstery, carpeting, wall covering, and ceiling materials. Accidental fires can produce similar or higher amounts of brominated dioxins and furans to their chlorinated congeners. Ash-laden runoff samples collected after the attack on the World Trade Center in USA in September 2001 and subsequent fire demonstrated the release into the environment of PBDD/Fs among other brominated and chlorinated compounds (21).

The potential formation of large amounts of by-products such as chlorinated and brominated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs and PBDD/Fs) is a major concern during fires. Firefighters are particularly exposed to these toxic compounds. They may be exposed to a wide range of toxic chemicals both during and while cleaning up after fires, including volatile organic compounds, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), brominated flame retardants, and various combustion by-products. PBDD/Fs were found in firefighters' serum sampled after a fire along with other polybrominated compounds, confirming that they were exposed to these pollutants in smoke during firefighting (29).

Open domestic waste burning and other uncontrolled waste combustion processes also are a rich source of these contaminants. A pioneering work on the determination of PBDD/F emission factors from open domestic waste burning was written by Gullett *et al.* (13). In their opinion, the origin of these PBDD/Fs may partly be explained by their presence as contaminants in the commercial PBDE flame retardant mixtures in existing (household) materials, but *de novo* synthesis can not be excluded (13, 36). Large open dumping sites of municipal and electronic waste cause severe dioxin related pollution and lead to soil and marine environment pollution (33). The presence of PBDD/Fs in the environment is associated with their transportability.

There is little data available on the environmental transport, distribution and transformation of PBDD/Fs. Their physicochemical properties suggest similar environmental transport and distribution to PCDDs/PCDFs. Released to the environment, they may be most readily distributed into carbon- or fat-rich compartments. Based on the similar high octanol/water partition coefficients calculated, their bioavailability is comparable to that of chlorinated dioxins. PBDDs and PBDFs seem to be poorly degradable by microorganisms (37).

There are also some other sources of brominated dioxins. They have been detected in textile manufacturing, where brominated flame retardants have been used. PBDD/Fs along with PCDDs/PCDFs have been detected in leaded petrol-fuelled ICE emissions, unleaded petrol-fuelled ICE emissions of

catalysed and uncatalysed vehicles, and in diesel engine emissions. Certain lower brominated PBDD congeners (tri- tetra-) may be also produced through biogenic formation *via* precursor pollutants in the marine environment and bioaccumulate in some marine species such as fish and shellfish (10). Several PBDD congeners were identified as natural products in red alga (*Ceramium tenuicorne*) and blue mussels (*Metilus edulis*) living in the Baltic Sea (15, 22).

Biological and toxic effects. Despite a lack of strong evidence of individual congeners' toxicity, it is generally assumed that the biological activity of brominated dioxins is equal to that of their chlorinated analogues. PBDDs/PBDFs share a common mechanism of action with PCDDs/PCDFs and other related halogenated aromatic hydrocarbons. Due to their stereo-chemical configuration and size, dioxins and several other polyhalogenated hydrocarbons bind to the aryl hydrocarbon receptor (AhR) and activate its signal transduction pathway. The receptor's role in mediating toxic and biological effects has been well documented in numerous studies (6, 28). The strength with which the contaminants bind to the receptor is proportional to the elicited toxicity, enhanced gene transcription, and enzyme activities mediated by the receptor. Binding to the cytosolic Ah receptor, which plays a central role in mediating 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) -like toxicity, was confirmed for several PBDD/Fs. Their receptor-binding affinities varied by several orders of magnitude but were comparable to those of their chlorinated analogues. Classic TCDD-like responses have also been measured *in vitro*, including enzyme induction, anti-estrogen activity in human breast cancer cells, and transformation of mouse macrophages into tumour cells. Structure-activity relationship studies on PBDD/Fs suggest a similar mode of biological activity to the chlorinated analogues, which is likely to be initiated by Ah receptor binding (2, 3, 23).

Toxicity studies that have been carried out on PBDD/Fs show biological responses comparable to the chlorinated dioxins. The biological effects in experimental animal models of several mammalian species including rats, mice, rabbits, guinea pigs, and monkeys, as well as fish were those classic for 2,3,7,8-TCDD: lethality, wasting, thymic atrophy, terato-genesis, reproductive defects, chloracne, immuno-toxicity, enzyme induction, decreases in T4 and vitamin A levels, and increased hepatic porphyrins. These effects of brominated congeners are found at similar low doses as 2,3,7,8-substituted PCDDs and PCDFs, indicating comparable potencies for the 2,3,7,8-brominated congeners (3).

Based likewise on limited data it can be assumed that dermal, gastrointestinal, and pulmonary uptake does not vary significantly between the chlorinated and brominated congeners, and the amount of absorption depends significantly on the number of bromine substituents. The toxicokinetics of PBDDs and PBDFs indicates properties comparable with the chlorinated congeners (tissue distribution, very slow metabolism, and excretion) (35). The World Health Organisation has published a broad review of the biological and toxic properties of PBDDs and PBDFs and concluded that brominated and chlorinated 2,3,7,8-substituted congeners elicit a similar spectrum of effects (37). Recently a review paper on exposure, the mechanism of action, the toxicity of the brominated analogues, and arguments for including these compounds in the existing WHO toxic equivalency factor (TEF) scheme was written by Van den Berg *et al.* (35).

Diabetes is a major threat to public health worldwide. Understanding the role of environmental chemicals in the development or progression of diabetes is an emerging issue in environmental health. Recently, Taylor *et al*. (32) assessed the epidemiologic literature for evidence of associations between different persistent organic pollutants and type 2 diabetes. They find the overall evidence sufficient for the positive association of some organochlorine POPs with type 2 diabetes. The strongest positive correlation occurs with such organochlorine compounds as polychlorinated biphenyls, dioxins, and dioxin-like chemicals.

Although the majority of toxicity data come from *in vivo* and *in vitro* studies in mammalian models, limited data is also available for fish. There is an increasing number of evidence that suggest that some of these compounds show dioxin-like toxicity in fish. The results of the study by Norman *et al.* (24) demonstrate that dietary exposure to sublethal concentrations of brominated dioxins may impair reproductive physiology in fish and induce AhR-regulated genes. The attention should be also paid to the fact that the halogenated contaminant burden in fish may cause toxic effects in fish populations and thus impact their status. The trend of a declining cod population (*G. morhua callarias*) in the Baltic Sea over the last several decades has coincided with its increased contamination and leads us to assume that the cod population may be suffering from AhR-related effects. The assumption can be justified by the extremely high concentrations of PCDD/Fs and DL-PCBs that have been observed in the liver of this species (25, 30). These can also be indicative of a contaminant burden in the gonads as maternal transfer of contaminants to gonads has been documented (5).

Relatively high levels from ng/g to µg/g of lipids of brominated dioxins (PBDDs) were detected in Baltic Sea biota, predominantly in the productive coastal zone of the Baltic Sea (15, 22).

Occurrence and exposure. Brominated dioxins and furans are major contaminants, both indoors and in the environment (13, 16, 29, 31, 33). In ambient air, PBDFs were found more frequently than PBDDs. Outdoor dust samples (mainly from motorways) also showed a predominance of PBDFs. Indoor air samples taken from rooms equipped with a number of operating electronic appliances (televisions and/or computer

monitors) showed the presence of PBDFs (tetra to hepta) at total concentrations ranging from 0.23 to 1.27 pg/m^3. PBDDs were not detected. The total concentration of PBDD/Fs was equal to that of PCDD/Fs in dust from computer rooms but in house dust the concentration was lower than that of PCDD/Fs. In river and marine sediment samples from an industrialized zone, tetra- to hexaBDFs were detected. Sediment from road drainage contained PBDFs but no PBDDs. Similarly, soil samples taken near a motorway contained monobromodibenzofurans (monoBDFs or MoBDFs) and dibromodibenzofurans (DiBDFs), but no PBDDs (37).

Over the last decade there has been accumulating evidence that PBDDs and PBDFs can be found in a variety of biological matrices in addition to their well-known presence in abiotic matrices such as fly ash. Nevertheless, the number of investigation reports on the occurrence of PBDD/Fs in food, fish and shellfish, and biological samples is small. The primary route of human exposure to dioxins and furans is from the diet. Fernandes *et al.* (12) investigated the presence of PBDD/Fs in variety of Irish foods over a few years. For calculation of the result they used current toxicity equivalency factors for 2,3,7,8-dioxins and furans as suggested by the World Health Organization (37). A number of different food matrices were analyzed: milk, eggs, fish (river and marine species), shellfish (molluscs and crustaceans), meat, offal, vegetable, and cheese. The matrices were selected to represent commonly consumed foods, but some of them, *e.g*. shellfish, are also good indicators of environmental contamination. Most of the analyzed foods showed the presence of at least some of these contaminants, but a higher detection frequency and relatively higher contaminant content were observed in samples of shellfish, fish, and liver. The observations on occurrence of these contaminants in commonly consumed foods support the requirement for their further investigation. The supporting toxicology is likewise required that would refine the estimates of this toxic content.

Due to the prevalence of 2,3,7,8- substituted PBDDs and PBDFs, particularly in the aquatic environment, it is not surprising that these compounds have been detected in seafood for human consumption (1, 5, 10, 18). Fish and seafood are generally recommended by nutritionists as healthy foods that provide a good source of protein. Many oily fish species contain nutrients such as omega-3 fatty acids, which may reduce the risk of heart disease and stroke. However, fish and shellfish can accumulate contaminants from the water in which they live or from the food they eat. Shellfish feed by filtering plankton from the current moving through their habitat. This feeding mechanism leads to the bioaccumulation of pollutants of anthropogenic and biogenic origin from the surrounding water even at a considerable distance from pollution sources. Fish and shellfish were also investigated for their accumulation of halogenated compounds. Concentrations of PBDDs in littoral fish and mussels were generally higher than their chlorinated analogues, and the PBDD concentrations in mussels increased between 1995 and 2003 (15).

PBDD/Fs were found in commonly consumed species of marine shellfish in the United Kingdom. Individual samples of Pacific oysters (*Crassostrea gigas*), native oysters (*Ostrea edulis*), mussels (*Mytilus edulis*), scallops (*Pecten maximus*), and cockles (*Cerastoderma edule*) were collected from different coastal regions between 2006 and 2007. PBDD/Fs were detected in most shellfish samples at varying concentrations depending on the species. Toxic equivalents (TEQ) were higher in scallop gonads than in mussels' (0.083 ng/kg compared to 0.055 ng/kg whole weight respectively), although mussels showed the more complete range of detectable congeners, particularly the PBDFs (27). Polybrominated dibenzofurans occurred more frequently and generally at higher levels than polybrominated dibenzo-*p*-dioxins except for 2,3,7-TriBDD, which was the predominant PBDD/F congener in oysters. According to Rose and Fernandes (27), this profile may reflect the environmental distribution of these compounds and the effects of removal mechanisms - degradation, selective uptake and metabolism.

Results from the UK shellfish survey showed congener profiles to be related to the hypothesis of biogenic formation and also showed significant levels of PBDFs. The congener profiles for the PBDFs (tri- to penta-brominated) had similarities to those observed for environmental samples, in that a wide range was observed. This congener profile suggests an origin from industrial activity and it is likely that the observed profile and levels are due to combustion processes such as incineration. The predominance of PBDFs over PBDDs has also been observed in other studies involving combustion. Thus it is likely that the PBDD/Fs observed in these samples reflect both types of sources, biogenically mediated as well as anthropogenic (15, 22, 27).

These compounds are also commonly found in the aquatic biota. Several PBDD congeners were identified as natural products in red alga (*Ceramium tenuicorne*) and blue mussels (*Mytilus edulis*) living in the Baltic Sea (15, 22). For many years the Baltic Sea has been contaminated by industrial emissions of POPs. Several studies emphasized the dominant importance of atmospheric deposition of these pollutants. Most organohalogen contaminants released over the years are still present in the aqueous environment and thus are accessible to marine organisms. Elevated concentrations of PCDDs, PCDFs, and dl-PCBs in some Baltic fish species (salmon, herring, and sprat) have been shown in several surveys in European member states (18, 25, 30). Recently the contamination profiles of PBDDs and PBDFs were described in the tissue of Baltic wild salmon and compared with

chlorinated dioxins, dibenzofurans, and PCBs. The toxic equivalents (WHO-TEQs) for analyzed PBDD/Fs were found to contribute about 2.1% of the total PCDD/F-PBDD/F-TEQ (0.074–0.142 pg TEQ/g^{-1} f.w.) (38). Although concentrations of persistent organic pollutants in the Baltic Sea have been declining in the last decades, they continue to be of concern due to high levels of polychlorinated dioxins and furans, biphenyls and brominated compounds in marine organisms (4, 9, 18, 25, 30). Human exposure and the health risk which can result from the consumption of contaminated fish are important issues. To protect consumers Commission Regulation 1259/2011 has been imposed. Recent studies from various countries indicate that PBDDs and PBDFs can contribute significantly to the total amount of TEQ in seafood items compared to their chlorinated congeners (1, 10, 12).

There is some quantitative information available on the exposure of the general human population and on special subpopulations. The human exposure to these compounds is confirmed by the presence of PBDD/Fs in blood and adipose tissue. Polybrominated dibenzofurans were detected in all human adipose tissue samples in a Swedish study, confirming their presence in the population there. The highest concentration was found for 2,3,7,8-TeBDF, ranging from 0.27 to 2.4 pg/g lipid, followed by 1,2,3,7,8-PeBDF (0.23–0.89 pg/g lipid), 2,3,4,7,8-PeBDF (0.44–0.54 pg/g lipid), and 2,7/2,8-DiBDF (0.19–0.30 pg/g lipid). These findings illustrate the importance of continuous monitoring of brominated compounds in humans and in the environment (19). Polybrominated dibenzo-p-dioxins and dibenzofurans were found in the serum of firefighters (29). Occupational exposure to PBDD/Fs has also been reported in adipose tissues of workers in a fire retardant factory. Generally, PBDFs were more abundant than PBDDs, and PBDF air concentrations were the highest at workplaces where PBDE-containing polymers were produced. In many samples, 2,3,7,8-substituted PBDFs and PBDDs were detectable. PBDD/F contamination was also found at the work area under the fume hood of a chemical laboratory (37).

The toxic equivalent factors (TEF) recommended by the WHO for PBDD and PBDF allow determination of the total combined toxicity in pg WHO-TEQ/g from parallel determination of chlorinated dioxins, furans, and DL-PCBs, and determination of the potential risk from these five groups of contaminants (the sum of PBDD, PBDF, PCDD, PCDF, and DL-PCB) (35). In industrial countries, PBDD/Fs are released from uncontrolled burning of BFR-containing wastes and the resulting emissions contribute substantially to total dioxin-like toxicity (13). In Japan, PBDFs are the major contributors to dioxin-like toxicity in house dust (31). In the UK, PBDD/Fs and PCDD/Fs contribute about 30% of the dioxin-like toxicity in food (27). A Swedish study of adipose tissue samples indicated that PBDD/Fs may contribute up to 14% of the total dioxin toxic equivalents (19). German authors also reported that the TEQ of PBDD/Fs may account for up to 12% of the dioxin-like toxicity in human milk (20).

Although toxicity to humans is unknown, it is expected that due to the common mechanism of action with chlorinated dioxins and furans these brominated compounds are also not neutral to human health. The scientific and environmental evidence has shown that at least certain brominated analogues of classic dioxins (PCDD/Fs) can also figure as environmental and human body contaminants (26). These compounds are polybrominated dibenzo-p-dioxins and polybrominated dibenzofurans, which are structurally analogous to their chlorinated counterparts.

Research methodology. Determination of certain bromoorganics is still an analytical challenge. Although there are a number of methods reported for the analysis of chlorinated dioxins, PCBs, and PBDEs, very few methods exist for the determination of PBDD/Fs (1, 8, 11, 12, 14). Highly sensitive, selective, and specific analytical methods (gas chromatography/mass spectrometry (GC/MS)) are required because of the large number of PBDD and PBDF congeners. Methodological problems related to thermal and photolytic degradation and low concentrations in food samples (pg/g of fresh weight) create additional difficulties. Therefore, the extension of existing analytical methods initially developed for PCDD/F is essential for determination of PBDD/F congeners. Most of the research is concentrated on adaptation of analytical methods routinely used for simultaneous analysis of ultra-trace levels of 2,3,7,8-substituted PBDDs/Fs and PCDDs/Fs. Clearly defined criteria for positive identification and a Quality Control/Quality Assurance system are essential elements of these methods.

Sampling, extraction, clean-up, and analysis for PBDDs/PBDFs mostly follow the methods and techniques currently used for PCDDs/PCDFs (11, 27). The large number of isomers in some homologous groups (Table) makes the separation and quantification of individual congeners very difficult. The analytical methodology uses $^{13}C_{12}$ labelled surrogates of the target compounds. Detection, quantification, and confirmation are usually performed by mass spectrometry (MS), as the only technique that shows sufficient selectivity to distinguish individual PBDDs/PBDFs from other halogenated compounds (*e.g.* PBDEs) also present in the sample. MS allows the determination of the number and type of halogens on the basis of characteristic isotope distribution patterns. Among the MS methods, high-resolution mass spectrometry (HRMS) is preferred owing to its highest selectivity. Isotopic dilution high resolution GC/HRMS is the most advanced analytical method of simultaneous separation and quantification of hundreds of substances at pg/g levels (17).

It is generally accepted that the vast majority of human exposure to chlorinated dioxins and PCBs is

through diet (over 90%). Recognizing this, EU regulations limiting the concentrations of chlorinated dioxins in food and animal feed came into force in July 2001 (4). With substantial improvements in analytic techniques over the past two decades, it has become possible to measure PCDDs and PCDFs in a congener-specific fashion down to the low parts per trillion levels. Similar regulations, controlling the occurrence of DL-PCBs were introduced at the end of 2006 and in 2011 for NDL-PCB (Reg. 1259/2011/EU and Reg. 252/2012/EU). These regulations also documented criteria governing the analytical methodology used for the determination of dioxins and PCBs. The criteria derived from the collective expertise and experience of specialist laboratories across the EU. The same criteria and similar guidelines should be used for the determination of PBDD/Fs as well (Fig. 3).

TEQ concept. Because term „dioxins" refers to such a broad class of compounds that vary widely in toxicity, the concept of toxic equivalence (TEQ) has been developed to facilitate risk assessment and regulatory control. Analytical results relating to all individual dioxin and dioxin-like PCB congeners of toxicological concern are expressed in terms of a quantifiable unit, namely the TCDD toxic equivalent (TEQ). The TEQ approach converts concentrations of various dioxin-like compounds (Fig. 4) into a single concentration that is toxicologically equivalent to the most toxic dioxin compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), using toxic equivalency factors (TEFs). At present, WHO-TEFs for human risk assessment of dioxins and dioxin-like compounds are based on the conclusions of the WHO-IPCS expert meeting, which was held in Geneva in June 2005 (34).

In 2011, a joint WHO and United Nations Environment Program (UNEP) expert consultation concluded with a recommendation for the possible inclusion of brominated analogues of the dioxin-like compounds in the WHO Toxicity Equivalency Factor (TEF) scheme. According to expert opinion, PBDDs, PBDFs, and some dioxin-like biphenyls (PBBs) in daily human background exposure may contribute significantly to the total dioxin toxic equivalencies (TEQs). However, the specific relative effect potencies (REPs) for PBDDs, PBDFs, and *non-ortho* DL-PBBs in mammals closely follow those of the chlorinated analogues, at least within one order of magnitude. Therefore, the use of similar interim TEF values for brominated and chlorinated congeners for human risk assessment was recommended. The review paper of Van den Berg *et al.* (35) shows the outcome of a joint WHO-UNEP expert consultation.

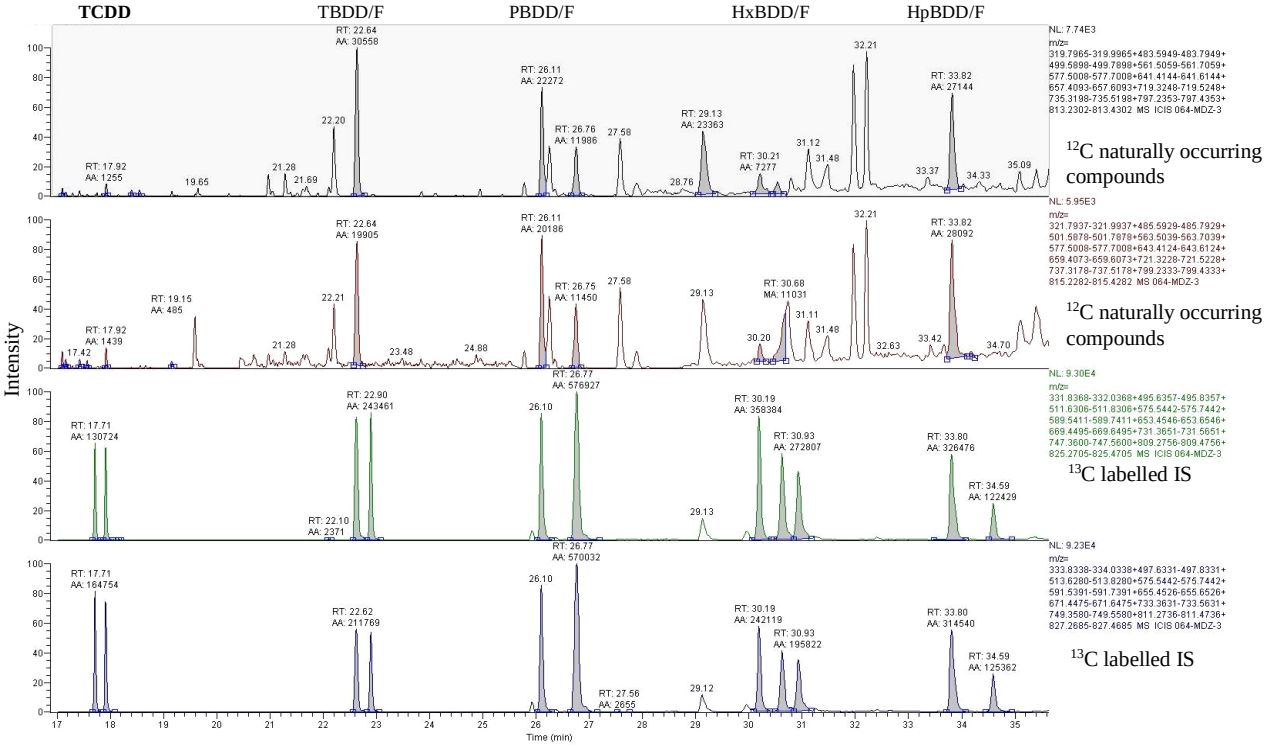


Fig. 3. Ion chromatogram showing PBDD/F mixture run on HRGC-HRMS (HRMS resolution >10 000)

$$TEQ = \sum_{i=1}^{7}\left(PCDD_i \times TEF_i\right) + \sum_{j=1}^{10}\left(PCDF_j \times TEF_j\right) + \sum_{k=1}^{12}\left(PCB_k \times TEF_k\right)$$

Fig. 4. Formula for determination TEQ of PCDD/Fs

It discusses the available information on exposure, mechanism of action, and toxicity of the brominated analogues of PCDDs, PCDFs, and PCBs, and provides arguments for including these compounds in the existing WHO TEF scheme. The authors claim that at present there is sufficient evidence to conclude that concentrations of 2,3,7,8-substituted PBDDs and PBDFs in food can contribute significantly to the total amount of TEQ intake in mammalian systems. Based on the present knowledge, the use of similar interim TEF values for brominated and chlorinated dioxin congeners for human risk assessment, as recommended by the WHO (WHO-TEF_{2005}), is suggested, creating the availability of studies on these compounds in the future.

Concluding remarks. The findings reported during last decade provide some evidence of the PBDD/Fs as an up-coming class of contaminants. Even limited data on the biological activity supports the hypothesis that these brominated compounds have a similar effect on health to their chlorinated relatives. The examination of these pollutants confirmed dioxin-like effects at low concentrations, suggesting their toxicological significance. Their biological activities make them logical subjects for environmental and toxicological investigation.

The mechanism of action and type of toxicity of 2,3,7,8-substituted PBDDs and PBDFs are also similar to their chlorinated analogues. At present, there is sufficient evidence to conclude that concentrations of 2,3,7,8- substituted PBDD/Fs in food can contribute significantly to the total amount of TEQ intake in mammalian systems. Knowing the common mechanism of biological action and toxic effects, it is reasonable to predict that their presence will incrementally add to the total dioxin body burden.

There are several factors which should be considered when assessing the human health risk of brominated dioxins and furans. The human exposure is confirmed by their reported occurrence in human tissues. The levels of human exposure to PBDD/Fs as a result of food consumption should be a subject of concern; therefore, one of the basic questions concerning this class of organic pollutants is: if they enter the food chain, what is the level of human exposure? Several methods of determination of brominated dioxins and furans are available in the literature. Removing methodological barriers advances us towards answers now to the above questions. Following the recommendation for the use of interim WHO-TEF_{2005} values for brominated and chlorinated dioxin congeners for human risk assessment allows us to do further research.

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