

Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs
3. Regulations and analytical methods
4. Future trends and recommendations

Notable links



Persistent Organic Pollutants in Food and the Environment

Overview of regulations, analysis and trends

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs
3. Regulations and analytical methods
4. Future trends and recommendations

Notable links

Persistent Organic Pollutants (POPs) are chemicals that have certain characteristics in common:

- tendency to remain intact for exceptionally long periods of time (up to many years)
- wide distribution throughout the environment as a result of natural processes involving soil, water and, most notably, air
- accumulation in the fatty tissue of living organisms including humans, and are found at higher concentrations at higher levels in the food chain
- toxic to both humans and wildlife

POPs have been synthesized to serve useful purposes such as dichlorodiphenyl-trichloroethane (DDT), which was used as an insecticide during World War II and saved thousands of people from death by malaria, or polychlorinated biphenyls (PCBs) that found wide application in the electrical industry. Concerns about POPs did arise in the 1960s, when they were detected in the environment, in wildlife and in humans. The Seveso incident in Italy in 1976, made the world aware of the existence of an extremely toxic, unintentionally generated POP, namely 2,3,7,8-tetrachlorodibenzo-para-dioxin (2,3,7,8-TCDD), which subsequently was extended to a whole family of dioxin-like POPs, including additional polychlorinated dibenzo-*p*-dioxins (PCDD), dibenzofurans (PCDF), and specific PCBs.

Being aware of the risks posed by POPs, the Stockholm Convention on Persistent Organic Pollutants was agreed in 2001 as a legally binding instrument, which aims to eliminate or to restrict the production and the use of POPs at a global level. It entered into force on 17 May 2004 and as of July 2017 has 181 participants.

Foreward by Heidelore Fiedler

Initially, the Stockholm Convention targeted twelve chemicals for reduction and eventual elimination. More importantly, the system to list additional chemicals having a POPs characteristic has shown to be successful and up to 2017, 16 more chemicals have been listed in the annexes A, B or C of the Convention. All of the 28 POPs covered by the Stockholm Convention are halogenated, *i.e.*, have fluorine, bromine or chlorine atoms in the organic molecule. Under the Stockholm Convention worldwide, countries are required to implement control measures for each of the chemicals and report progress towards elimination or reduction of the POPs. The convention also includes monitoring and chemical analysis of the POPs in the environment and in humans. Thus, the identification and quantification of the amount of POPs in emissions, commodities/products, in the environment or humans is an important activity for each country.

This booklet contains information on the history of POPs, the implications of their early occurrence, and today's analytical testing methods for their accurate determination in chemical laboratories as an introduction to this global issue.

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As one of the founding members of the Stockholm Convention, her research focus is on environmental chemistry from identification and quantification of sources of (persistent organic) pollutants through environmental fate and transport to human exposure and effects in humans and the environment.



Persistent Organic Pollutants

- 1. POPs: What are POPs and why do we need to talk about them?
- 2. Introduction of classified POPs
- 3. Regulations and analytical methods
- 4. Future trends and recommendations

Notable links

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

- Historical Background 5
- Chemicals classified as POPs 7
- Environmental contamination 8
- Dispersal of POPs throughout the environment 9
- Classification of the original "Dirty Dozen" from the Stockholm Convention 10
- The new POPs 11

2. Introduction of classified POPs

- Pesticides 12
- Industrial chemicals 14
- By-products / Unintentionally Produced Products 16

3. Regulations and analytical methods

- PCDD/Fs and PCBs 17
- Non-dioxin like PCBs (NDL-PCB) 21
- Polybrominated diphenyl ethers (PBDEs) 23
- Perfluoroalkylated substances (PFOAs and PFOS) 24
- Pesticides 25

- Hexabromocyclododecane (HBCD) 26
- Short-chain chlorinated paraffins (SCCPs) 27
- Isotope dilution method 28
- Monitoring and surveillance of POPs 29
- 4. Future trends and recommendations** 31
- Noteable links** 34



What are POPs and why do we need to talk about them?

Historical Background

Silent Spring

The publication of *Silent Spring* by Rachel Carson in 1962 brought to public attention the detrimental effects on the environment of the indiscriminate use of organochlorine pesticides. The emotive title of this book rightly publicized a situation where unrestricted use of pesticides was resulting in a widespread extinction of species, including birds. Inevitably, *Silent Spring* was strongly criticized by chemical companies over many years, with media attacks not only on the book, but also the credibility of the author. Despite these attacks, later scientific research backed her claims, and the negative side effects of pesticides are now well documented and proven beyond doubt. The work of Rachel Carson is seen as instrumental in establishing an environmental movement and in the creation of regulatory bodies responsible for environmental protection such as the [United States Environmental Protection Agency](#) (U.S. EPA).

Stockholm Convention

During the 1970s and 1980s, actions concerning the control of pesticides were taken, mostly at national level, and these actions included banning the widespread use of DDT as an agricultural pesticide in many countries. In May 1995 the Governing Council of the [United Nations Environment Programme](#) (UNEP) requested in its decision 18/32 that an international assessment process be undertaken of an initial list of 12 POPs and that the Intergovernmental Forum on Chemical Safety (IFCS) develop recommendations on international action for consideration by UNEP Governing Council and World Health Assembly no later than 1997. The IFCS concluded in June 1996 that available information was sufficient to demonstrate that international action, including a global

legally binding instrument, was required to minimize the risks from the 12 POPs through measures to reduce and/or eliminate their emissions or discharges. The UNEP Governing Council in its decision 19/13C from February 1997 invited UNEP to prepare for and convene an intergovernmental negotiating committee (INC), with a mandate to prepare an international legally binding instrument for implementing international action initially beginning with the 12 POPs and requested that the INC establish an expert group to develop criteria and a procedure for identifying additional POPs as candidates for future international action. This process was concluded in 2001 and became known as the [Stockholm Convention on Persistent Organic Pollutants](#). This legally binding global treaty on POPs entered into force in May 2004 and as of July 2017, has 181 parties.

Notably, governments that have not ratified the convention include the United States of America, Israel, Malaysia, and Italy.

The objective of this international agreement is to protect human health and the environment from the negative effects caused by POPs, by eliminating and/or restricting their use. Initially, 12 substances were controlled, with the list being progressively expanded based on new knowledge through subsequent intergovernmental meetings.

STOCKHOLM CONVENTION

The objective of this international agreement is to protect human and animal health from the impact of POPs by eliminating and/or restricting use of these man-made chemicals that are persistent in the environment and damaging to human and animal health.

Initially, 12 substances were controlled, with the list being progressively expanded on the basis of new knowledge through subsequent intergovernmental meetings.



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

Historical Background

Chemicals classified as POPs

Environmental contamination

Dispersal of POPs throughout the environment

Classification of the original "Dirty Dozen" from the Stockholm Convention

The new POPs

2. Introduction of classified POPs

3. Regulations and analytical methods

4. Future trends and recommendations

Notable links

Historical examples of world-wide incidents involving POPs

Pre-dating the Stockholm Convention, from the late 1960s to the present time, there have been numerous incidents, where through accidents or even deliberate adulteration there has been human and animal exposure to POPs (principally to PCBs and dioxins). Some of these incidents are shown in the table below.

Event	POPs	Impact	Country	Year
Publication of Silent Spring	Organochlorine pesticides	Public awareness of long-term effects of pesticides on animal and human health	USA	1962
Agent Orange (2,4-T & 2,4,5-T) used in Vietnam war	TCDD contaminant in defoliant	TCDD aerial exposure and soil contamination – health effects	Vietnam	1961-1971
Fire at Coalite chemical plant (Bolsover)	Dioxins formed during fire	79 workers treated for chloracne – 1991 soil and milk contamination	UK	1968
Yushō disease from factory contaminated rice oil	PCBs in cooling fluid	400,000 poultry died and 14,000 people had health effects	Japan	1968
U.S. EPA introduced a ban on the use of DDT	DDT	Use of DDT banned for agricultural purposes	USA	1972
Seveso – industrial accident releasing 6 tons of chemicals	Dioxins formed by chemical reaction	447 people with chloracne – 80,000 animals affected	Italy	1976
Yu-cheng – PCB heat-transfer medium leak into rice oil	PCBs, PCDFs, PCQs from heat medium	2,000 people affected with symptoms such as chloracne	Taiwan	1979
Hazardous waste disposal – Times Beach, Missouri	Dioxins	This Missouri town was so polluted the EPA just bought it and incinerated all the houses	USA	1983
Citrus pulp (CP) pellets for animal feed	Dioxins in lime added to peel before drying	90,000 metric tons of CP destroyed	Brazil	1998
Oil accidentally added to recycled fat for animal feed	PCBs & dioxins	2,500 farms affected – direct cost €1 billion and indirect cost €3 billion	Belgium	1999
Potatoes sorted in kaolinic clay bath	Dioxin contamination of clay	Potatoes used in animal feed resulting in milk contamination	Netherlands	2004
RASFF identified contaminated guar gum	PCBs & PCP	Recall of guar gum edible thickening agent	India	2007
Adulteration of pig feed with waste oil	Dioxins & PCBs	PCBs and dioxins in pork – €100 million financial losses	Ireland	2008
Adulteration of feed with contaminated waste cooking oil	Dioxins & PCBs	PCBs and dioxins in meat – restrictions on 5,000 farms	Germany	2010

Accidents / Episodes

Agent Orange's long legacy for Vietnam and veterans

One of the most notorious episodes concerning POPs was the use of a defoliant called Agent Orange in the Vietnam War, which was found to be contaminated with dioxins.

The widespread use of this defoliant led not only to exposure of the local population and US troops to dioxins, but also gave rise to long-term contamination of soil.

Another well-known incident was the Seveso Disaster, which was an industrial accident that occurred in 1976 in a chemical manufacturing plant close to Milan. Around 6 tons of chemicals containing 1 kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were released into the air over an 18 km² area. Within days, 3,300 poultry birds and rabbits were found dead, and subsequently the emergency slaughtering of 80,000 animals was carried out to prevent TCDD from entering the food chain. Fifteen children were hospitalized with skin inflammation and 447 people were found to suffer from skin lesions or chloracne.

In the longer term, a zone where TCDD concentrations in the soil exceeded 50 µg/m² was completely evacuated and fenced off. This area has now been remediated and is accessible to the public, but there are still some highly contaminated areas left, that need to be cleaned.

Incidents in Japan and Taiwan occurred when PCBs used in coolant/heating systems in food factories accidentally leaked into the edible rice oils being produced. It was not until human poisoning incidents were reported that the failure was detected.

Many of the food-related POPs incidents that have occurred over the past 20 years have only been detected because of routine monitoring of PCDD/PCDF, and later PCBs in cow's milk, then traced back to a component of animal feed. It has been the persistence of POPs in the environment that has triggered action. For example, it was more than 20 years after the fire at the Coalite factory in the UK that dioxins were detected in milk from farms near the original accident.

There is now a good understanding of the issues related to organochlorine pesticides, PCDD/Fs and PCBs and considerable progress has been made in reducing environmental contamination and human exposure. In contrast, the level of knowledge is much lower concerning the health effects of newer brominated and fluorinated POPs which have been used extensively in industrial applications.



The long-term persistence of dioxins in the environment resulted in dioxin contamination of clay and lime, subsequently used in processing potatoes and citrus peel, which led to successive contamination.

1. POPs: What are POPs and why do we need to talk about them?

Historical Background

Chemicals classified as POPs

Environmental contamination

Dispersal of POPs throughout the environment

Classification of the original "Dirty Dozen" from the Stockholm Convention

The new POPs

2. Introduction of classified POPs**3. Regulations and analytical methods****4. Future trends and recommendations**

Notable links

POPs can be grouped into pesticides, industrial chemicals or unintentional chemicals (contaminants) formed during manufacture (impurities) or in thermal processes. Present POPs are all halogenated, *i.e.*, chlorinated, brominated or fluorinated. Some POPs are single compounds such as aldrin or hexachlorobenzene, and others are groups of chemicals such as toxaphene, PCBs, PCDDs or PCDFs.

The common features of chemicals that are classified as POPs are:

- all synthetic (man-made) organic chemicals
- persistent in the environment
- susceptible to long-range transport and therefore lead to global pollution
- have well-established toxicity

Although only twelve chemicals or classes of chemicals are targeted by the Stockholm Convention, more chemicals are under consideration for extending the convention coverage.

Chemicals classified as POPs



1. POPs: What are POPs and why do we need to talk about them?

Historical Background

Chemicals classified as POPs

Environmental contamination

Dispersal of POPs throughout the environment

Classification of the original "Dirty Dozen" from the Stockholm Convention

The new POPs

2. Introduction of classified POPs

3. Regulations and analytical methods

4. Future trends and recommendations

Noteable links

POPs have historically entered the environment through deliberate use as agrochemicals or as impurities in agrochemicals. Decades later they persist and can still be found in the food chain. PCBs were originally manufactured for specific uses such as coolants and insulating fluids for transformers and capacitors, hydraulic fluids, lubricating and cutting oils. They were also used in carbonless copy paper and as plasticizers in paints and cements, stabilizing additives in flexible PVC coatings of electrical cables and electronic components, pesticide extenders, reactive flame retardants, and sealants for caulking and adhesives.

Poor waste disposal practices for closed sources of PCBs, incineration of PCB-containing materials, and leaching from materials from landfill sites into water sources have led to widespread environmental contamination with PCBs.

PCDDs and PCDFs are not deliberately manufactured as they have no common uses, and therefore they exist only as by-products of industrial processes such as bleaching paper pulp and pesticide manufacturing. An important source of aerial contamination with dioxins has been combustion processes such as waste incineration, particularly involving chlorine-containing waste such as PVC. Dioxins have also been released through industrial fires and other accidents where large-scale emissions have occurred, rendering highly contaminated sites such as an area around Seveso, Italy.

POPs may be transported over long distances far from their original sources. Transport can occur through long-range air dispersion, through accumulation in migratory species, and also through human activities such as transport of equipment or waste containing POPs.

Environmental contamination



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

- Historical Background
- Chemicals classified as POPs
- Environmental contamination
- Dispersal of POPs throughout the environment
- Classification of the original "Dirty Dozen" from the Stockholm Convention
- The new POPs

2. Introduction of classified POPs

3. Regulations and analytical methods

4. Future trends and recommendations

Noteable links

The schematic illustrates how particulate and gaseous emissions from incineration are transported by air masses and then deposited long distances away by rainfall, thereby contaminating distant bodies of soil and water. The dispersion through water contamination progressively leads to global contamination of the environment and these POPs become ubiquitous environmental contaminants.

When POPs are released into the environment, they are absorbed by the fatty tissues of plants and animals. They become increasingly concentrated in those tissues through a process called bio-accumulation. The concentration also biomagnifies, leading to increases by hundreds and even millions of times, as organisms exposed to POPs are eaten by their predators. This allows POPs to move through the different links in food chains and become more highly concentrated in species that are at a higher level in food chains, such as fish, predator birds, mammals, and human beings. With POPs therefore, we see widespread dispersal in the environment leading to global contamination, the long-term physicochemical stability of POPs, and resulting bioaccumulation and magnification in food chains.

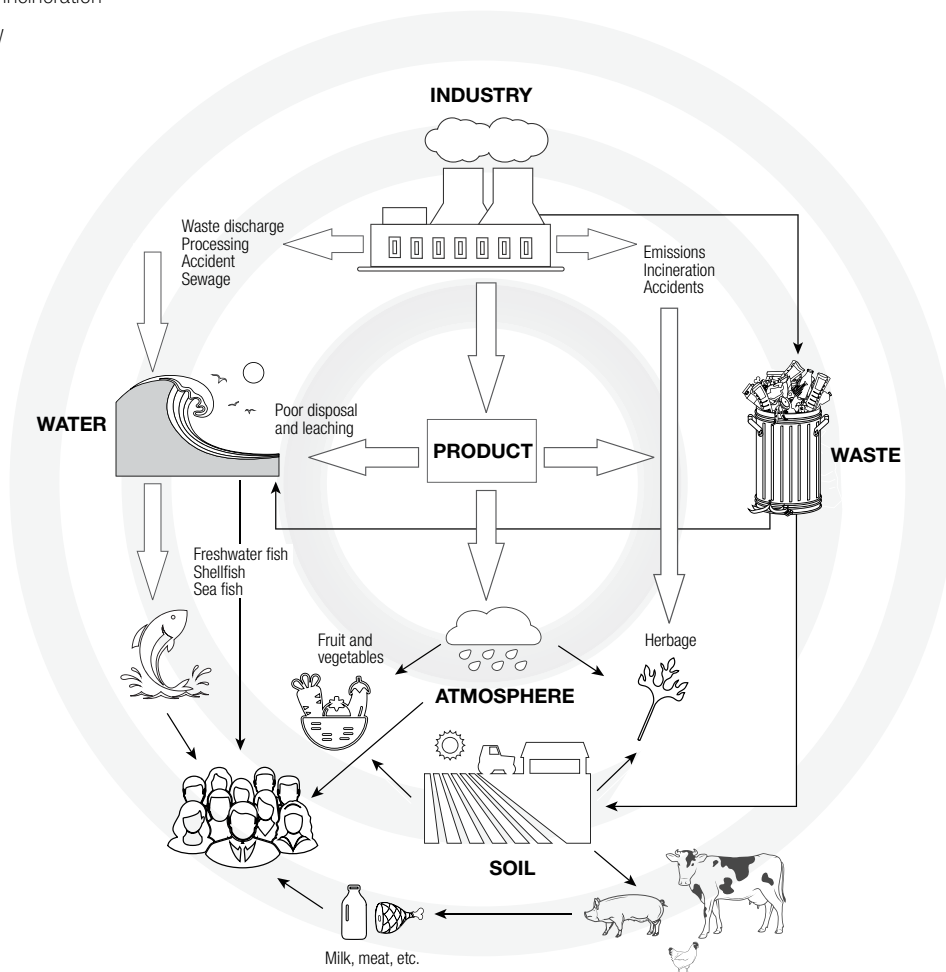


Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

Historical Background

Chemicals classified as POPs

Environmental contamination

Dispersal of POPs throughout the environment

Classification of the original "Dirty Dozen" from the Stockholm Convention

The new POPs

2. Introduction of classified POPs

3. Regulations and analytical methods

4. Future trends and recommendations

Notable links

Classification of the original "Dirty Dozen" from the Stockholm Convention



The characteristics of POPs are that they are all synthetic, entirely man-made chemicals, either intentionally or non-intentionally produced/released. All POPs have a remarkable stability to physical and biological effects with a consequence that they are highly persistent in the environment for decades or even hundreds of years. POPs are also susceptible to long-range transport in the environment leading to global pollution even in areas remote from the original source of contamination. Most POPs are lipophilic, and therefore tend to remain in fat-rich tissues and bioaccumulate. This results in biomagnification as POPs enter the food chain, accumulating in larger animal species as they consume smaller ones in the chain. Both the acute and chronic toxicity of exposure to POPs is well characterized, but long-term effects on human health such as carcinogenicity may take decades to become evident.

POPs are grouped into three categories: pesticides, industrial chemicals, and by-products. They are listed in three different annexes:

- Annex A (Elimination) – Parties must take measures to eliminate the production and use of the intentionally produced chemicals.
- Annex B (Restriction) – Parties must take measures to restrict the production and use of the listed chemicals.
- Annex C (Unintentional production) – Parties must take measures to reduce the unintentional releases of listed chemicals.

Chemicals	Pesticide	Industrial chemical	By-product	Annex
Aldrin	X			A
Chlordane	X			A
DDT	X			B
Dieldrin	X			A
Endrin	X			A
Heptachlor	X			A
Mirex	X			A
Toxaphene	X			A
Hexachlorobenzene (HCB)	X	X		A
Polychlorinated biphenyls (PCB)		X	X	A
Polychlorinated dibenzo-p-dioxins (PCDD)			X	C
Polychlorinated dibenzofurans (PCDF)			X	C



1. POPs: What are POPs and why do we need to talk about them?

Historical Background

Chemicals classified as POPs

Environmental contamination

Dispersal of POPs throughout the environment

Classification of the original "Dirty Dozen" from the Stockholm Convention

The new POPs

2. Introduction of classified POPs

3. Regulations and analytical methods

4. Future trends and recommendations

Notable links

The new POPs

There have been several meetings of the conference of the parties of the Stockholm Convention. At these meetings, several amendments to the initial Stockholm Convention list have been discussed and agreed upon. The following new POPs have been agreed, sorted to the list, and categorized in the same way as the initial twelve.

Chemical	Pesticide	Industrial chemical	By-product	Annex	Year
Alpha hexachlorocyclohexane	X			A	2009
Beta hexachlorocyclohexane	X			A	2009
Chlorodecone	X			A	2009
Hexabromobiphenyl		X		A	2009
Hexabromocyclododecane		X		A	2013
Hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromodiphenyl ether)		X		A	2009
Hexachlorobutadiene		X		A and C	2015 / 2017
Lindane	X			A	2009
Pentachlorobenzene	X	X	X	A and C	2009
Pentachlorophenol and its salts and esters	X			A	2015
Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride		X		B	2009
Polychlorinated naphthalenes		X	X	A and C	2015
Technical endosulfan and its related isomers	X			A	2011
Tetrabromodiphenyl ether and pentabromodiphenyl ether (commercial pentabromodiphenyl ether)		X		A	2009
Decabromodiphenyl ether (commercial mixture, c-decaBDE)		X		A	2017
Short-chain chlorinated paraffins		X		A	2017



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

Pesticides

Industrial chemicals

By-products / Unintentionally produced products

3. Regulations and analytical methods

4. Future trends and recommendations

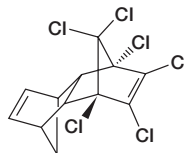
Notable links

Pesticides are a group of many different substances that are used to control pest and weeds. They can be classified by their target organism; for example, fungicides are used to eliminate or prevent fungal infestation. Other examples are algicides (algae), herbicides (plants), insecticides (insects), and rodenticides (rodents). Another system for classification of pesticides is to group them in accordance with their chemical structure, *e.g.*, carbamate, organophosphate (OP), and organochlorine (OC). All the POPs pesticides that are listed by the Stockholm Convention are organochlorine pesticides (OCPs).

The following short profiles of the Stockholm Convention pesticides show their molecular structures, and provide some information about the target organism.

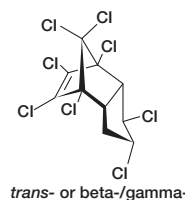
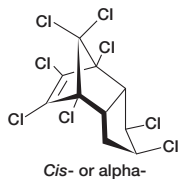
ALDRIN

Aldrin's chemical composition is $C_{12}H_8Cl_6$. It is applied to soils to kill insects such as termites and grasshoppers. Aldrin also affects birds, fish, and human health.



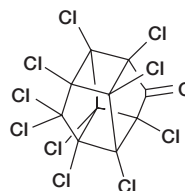
CHLORDANE

Chlordane appears in the form of two isomers, cis (alpha) and trans (beta/gamma). The chemical composition is $C_{10}H_6Cl_8$. It was used extensively to control termites.



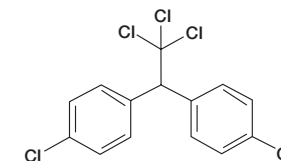
CHLORDECONE

Chlordecone is also known as Kepone and has the following chemical composition: $C_{10}Cl_{10}O$.



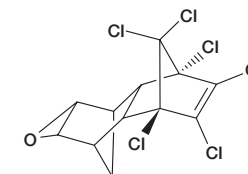
DDT

DDT was used to control malaria and typhus in the 1940s and the Nobel Prize in Physiology or Medicine in 1948 was awarded to Paul Hermann Müller for his "discovery of the high efficiency of DDT as a contact poison against several arthropods." The two DDT isomers are *p,p'*-DDT and *o,p'*-DDT. Impurities of technical mixtures of *p,p'*-DDT are *o,p'*-DDT, *p,p'*-/*o,p'*-DDE and *p,p'*-/*o,p'*-DDD. The chemical composition of DDT: $C_{14}H_9Cl_5$; DDE: $C_{14}H_8Cl_4$; and DDD: $C_{14}H_{10}Cl_4$.



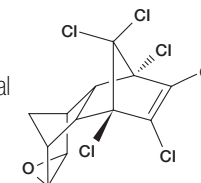
DIELDRIN

Dieldrin was used as insecticide and is closely related to aldrin, as aldrin is used as base for dieldrin. The chemical composition is $C_{12}H_8Cl_6O$.



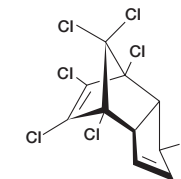
ENDRIN

Endrin is a stereoisomer of dieldrin, so it has the same structural composition ($C_{12}H_8Cl_6O$). It was mainly used as insecticide, but it is also effective for rodents and poisonous to fish.



HEPTACHLOR

Heptachlor was used as insecticide to kill soil insects and termites. $C_{10}H_5Cl_7$ is the chemical composition.



HEXACHLOROBENZENE

Hexachlorobenzene (HCB) is a fungicide and has the chemical composition of C_6Cl_6 . HCB is also listed as industrial chemical and by-product.

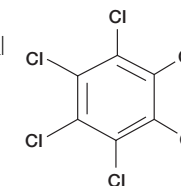


Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

Pesticides

Industrial chemicals

By-products / Unintentionally produced products

3. Regulations and analytical methods

4. Future trends and recommendations

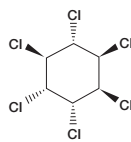
Notable links



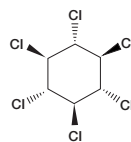
HEXACHLOROCYCLOHEXANE (ALPHA, BETA, AND GAMMA)

Hexachlorocyclohexane has several isomers and three of them are listed as POPs.

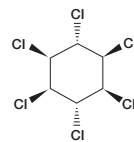
Lindane was used as an agricultural insecticide and as a pharmaceutical product. Due to the production process, alpha- and beta-HCH are byproducts of lindane and effective pesticides. All have the chemical composition of $C_6H_6Cl_6$.



Alpha



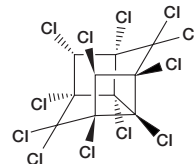
Beta



Gamma (Lindane)

MIREX

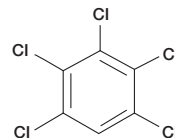
Mirex, with the chemical composition of $C_{10}Cl_{12}$, was used as insecticide to control fire ants and additionally was used as flame retardant.



PENTACHLOROBENZENE

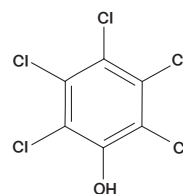
Pentachlorobenzene (PeCB) was used in different manners.

As pesticide, it was effective as a fungicide. Additionally, it was used as an additive to products that contained PCBs and as flame retardant. C_6HCl_5 is the chemical composition of PeCB. PeCB is also listed as industrial chemical and byproduct.



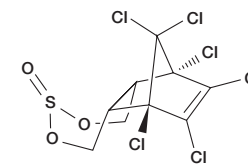
PENTACHLOROPHENOL AND ITS SALTS AND ESTERS

Pentachlorophenol (PCP) was first produced in the 1930s and used under many trade names. Due to its fungicidal effect, it was used for wood preservation. During the chlorination of phenol to create PCP, many contaminants are produced unintentionally. One of these contaminants is polychlorinated dibenzo-p-dioxins. The chemical composition is C_6Cl_5OH .



TECHNICAL ENDOSULFAN AND ITS RELATED ISOMERS

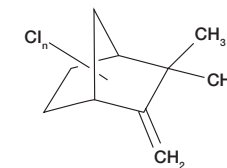
Endosulfan ($C_9H_6Cl_6O_3S$) is an insecticide that was developed in the early 1950s.



TOXAPHENE

To produce toxaphene, camphene ($C_{10}H_{16}$) is chlorinated.

The final product is a mixture with chemical composition from $C_{10}H_{11}Cl_5$ to $C_{10}H_6Cl_{12}$. It was used as pesticide.



Depending on the target matrix, target analytes and target residue levels of different analytical methods are available. For food samples, the most common extraction techniques are "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) and automated solvent extraction (ASE). Depending on the target analytes, gas chromatography (GC) or a liquid chromatography (LC) separation is performed followed by a mass spectrometer measurement. Performance and validation criteria for pesticide methods for food and feed samples are laid down in **Document No. SANTE/11945/2015** "Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed." As the guidance document is under continuous review, check for the latest version at www.crl-pesticides.eu.

In this white paper, risk assessment or regulations for organochlorine pesticide POPs are not covered, as these have been reviewed in depth in a separate white paper. Suffice it to say that EU Regulations 396/2005 and 1107/2009 set out maximum residue limits (MRLs) for pesticide residues and these are usefully compiled in a Pesticide MRL database.

For other sample matrices, such as environmental samples like soil, sediment, water, etc., other methods can be used. Most of the POPs pesticides are covered by US EPA Method 1699 "Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS" from December 2007.

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

Pesticides

Industrial chemicals

By-products / Unintentionally produced products

3. Regulations and analytical methods

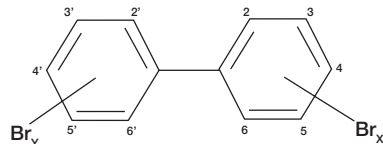
4. Future trends and recommendations

Noteable links

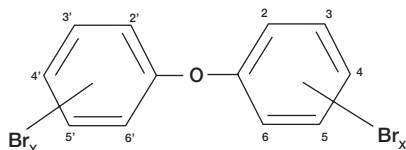
The industrial chemicals that are listed in the Stockholm Convention are produced for their specific characteristics. Many of them have been used as flame retardants (like HBB and the PBDEs), others have been used as dielectric fluid (like PCBs). All the compounds are halogenated, so they all are fluorinated, chlorinated, or brominated.

HEXABROMOBIPHENYL (HBB)

Hexabromobiphenyl has been sold under the trade name of Firemaster® as flame retardants. There are 42 isomers of hexabromobiphenyl. The chemical composition of all HBB isomers is $C_{12}H_4Cl_6$. The dominant one is 2,2',4,4',5,5'-hexabromobiphenyl, or PBB#153.



DECA-, HEXA-, HEPTA-, TETRA-, AND PENTA-BROMODIPHENYL ETHER (PBDE)

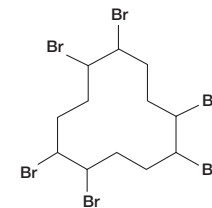


The PBDEs (tetrabromodiphenyl ethers and pentabromodiphenyl ethers) occur as two homolog groups with 42 tetrabrominated isomers and 46 pentabrominated isomers. Similarly, hexabromodiphenyl ethers and heptabromodiphenyl ethers occur as two homolog groups with 42 hexabrominated isomers and 24 heptabrominated isomers. Decabromodiphenyl is fully brominated ($C_{12}Cl_{10}O$) and a single isomer. The commercial decaBDE also consists of some nonabromo isomers. The generalized structure of a PBDE contains 1–10 bromine substituents. The chemical stability of the PBDE congeners vary with the individual structure, but in general, PBDE congeners with a low number of bromine substituents and those with nine and ten bromine substituents are the most vulnerable, while PBDE congeners with four to eight bromine substituents show the highest stability.

PBDE congeners may undergo photolysis and reductive debromination. Based on the physicochemical and reactivity of PBDEs, they are generalized as persistent even though individual PBDE congeners show differences in degrees of persistency due to their structures.

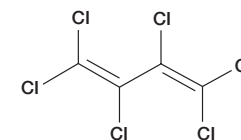
HEXABROMOCYCLODODECANE

Hexabromocyclododecane (HBCD or HBCDD) is used as a flame retardant especially with polystyrene foam as thermal insulation in buildings. There are 16 possible stereoisomers, but the main compounds used as flame retardants are α -HBCD, β -HBCD, and γ -HBCD.



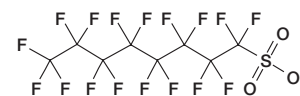
HEXACHLOROBUTADIENE

Hexachlorobutadiene (HCBd) can be used as hydraulic liquid, solvent, and cooling liquid for transformers. Mainly an unintentional contaminant

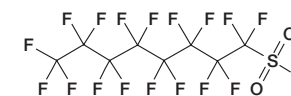


PERFLUOROCTANE SULFONIC ACID (PFOS), ITS SALTS, AND PERFLUOROCTANE SULFONYL FLUORIDE (PFOSF)

The terminology for **perfluorinated compounds (PFCs)** can be confusing as numerous acronyms are used for the various classes of compounds – **PFASs** refer to per- and polyfluoroalkyl substances, **PFCAs** to perfluorocarboxylic acids, **PFSAs** to perfluoro-sulfonic acids, and **PFAA** to perfluoroalkyl acids. Acronyms are also used for specific compounds such as perfluorobutanoic acid (**PFBA**), perfluorohexanoic acid (**PFHxA**), perfluorooctanoic acid (**PFOA**), perfluorononanoic acid (**PFNA**), perfluorobutane sulfonate (**PFBS**), and perfluorooctane sulfonate (**PFOS**).



PFOS: $C_8HF_{17}O_3S$



PFOSF: $C_8F_{18}O_2S$

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

Pesticides

Industrial chemicals

By-products / Unintentionally produced products

3. Regulations and analytical methods

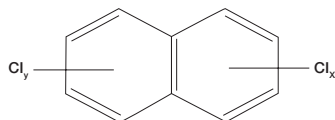
4. Future trends and recommendations

Noteable links

PFAAs have been used since the 1950s in numerous commercial applications, including as surfactants, lubricants, paper and textile coatings, polishes, food packaging, and fire-retarding foams. Perfluorooctane sulfonates or perfluorooctanesulfonic acid (PFOS) are fluorosurfactants, which are key ingredients in the fabric protector Scotchgard® and numerous stain repellents. PFOS is a single anionic compound with one linear and many-branched isomers. PFOS is structurally different from most of the other POPs in that it is based on a linear backbone rather than a ring structure and also contains some functionality in the sulfonic acid group.

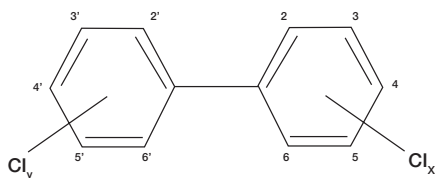
POLYCHLORINATED NAPHTHALENES

The polychlorinated naphthalenes (PCNs) are mixtures of up to 75 components. They have been used as isolating coats for electric cable and sealings.



POLYCHLORINATED BIPHENYLS

The polychlorinated biphenyls (PCBs) are sometimes referred to under their trade names for different mixtures. The trade name Aroclor is followed by a 4-digit number for which the first two digits refer to the number of carbon atoms in the biphenyl skeleton (for PCBs this is 12); the second two numbers indicate the percentage of chlorine by mass in the mixture. Thus, Aroclor 1260 has 12 carbon atoms and contains 60% chlorine by mass.



Based on the basic two benzene ring structure for PCBs, there are 209 possible congeners (common structures), where one to eight chlorines are substituted into the rings in different positions.

Polychlorinated biphenyls (PCBs) can be divided into different groups according to their biochemical and toxicological properties. Non-ortho and mono-ortho substituted PCBs show toxicological properties that are similar to PCDD/Fs. They are therefore often termed 'dioxin-like PCBs' (DL-PCBs). Most other PCBs do not show dioxin-like toxicity. The DL-PCBs have been included in the toxic equivalence concept of the PCDD/Fs.

SHORT-CHAIN CHLORINATED PARAFFINS (SCCP)

SCCPs are mixtures of polychlorinated alkanes. The carbon chains of SCCPs are from C10 to C13 with a variation of the chlorine degree between 30% and 70% of mass fraction. There are thousands of combinations possible and present in the commercial mixtures.

Chlorinated paraffins have a wide range of uses, for example, as flame retardants, lubricants, additives for sealants, and many others. For some applications, the chlorinated paraffins have replaced the PCBs.



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

Pesticides

Industrial chemicals

By-products / Unintentionally produced products

3. Regulations and analytical methods

4. Future trends and recommendations

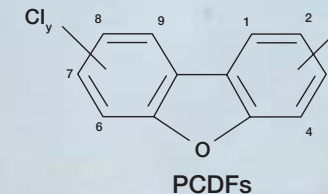
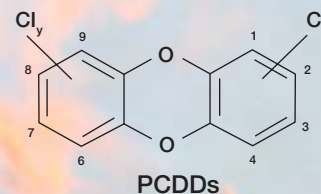
Noteable links

By-products / Unintentionally produced products

By-products have been created unintentionally and were not the target product. Usually, they do not cause trouble, except when they are much more toxic than the target product or the amount of by-product is significantly high. By-products can occur during chemical synthesis, like the PCDD impurities of PCP production, or during combustion processes, like incomplete waste incineration.

**POLYCHLORINATED DIBENZO-P-DIOXINS (PCDD) AND
POLYCHLORINATED DIBENZOFURANS (PCDF)**

The polychlorinated dibenzodioxins (dioxins) have two benzene rings with eight possible positions for chlorine substitution, giving rise to 75 possible congeners; the polychlorinated dibenzofurans (dibenzofurans) have the possibility of 135 congeners. It is important to note that not all PCDDs or PCDFs have equal toxicity, so individual congeners need to be determined rather than total concentrations.



To aid in risk assessment, a system of toxic equivalence factors (TEFs) has been established for seven dioxin congeners, 10 dibenzofurans, and 12 PCBs. The reference congener is the most toxic dioxin, which is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and by definition has a TEF of 1.0. To obtain the number of grams-TEQ of a dioxin/dibenzofuran/PCB mixture, the mass of each compound in the mixture is multiplied by its TEF and then the individual TEQs are summed to determine the TEQ. Similarly to dioxins and dibenzofurans, there are TEFs for DL-PCBs, which are typically 0.00003–0.0001, but PCB-126 is the single PCB with the highest TEF of 0.1.





Table of Contents

Persistent Organic Pollutants

- 1. POPs: What are POPs and why do we need to talk about them?
- 2. Introduction of classified POPs

3. Regulations and analytical methods

- PCDD/Fs and PCBs
- Non-dioxin like PCBs (NDL-PCB)
- Polybrominated diphenyl ethers (PBDEs)
- Perfluoroalkylated substances (PFOAs and PFOS)
- Pesticides
- Hexabromocyclododecane (HBCD)
- Short-chain chlorinated paraffins (SCCPs)
- Isotope dilution method
- Monitoring and surveillance of POPs

4. Future trends and recommendations

Noteable links

It should be noted that TEFs were established by WHO in 1998, and then revised in 2005, which resulted in some differences in values. It has, therefore, become customary when reporting TEQs to indicate whether calculated as TEQWHO98 or TEQWHO05. The 1998 values are still used in some instances as this has been the basis of regulatory limits, which have not been updated.

PCDD/F	Toxic Equivalency Factor (TEFWHO98)	Toxic Equivalency Factor (TEFWHO05)
Dioxins		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PnCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
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
Furans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PnCDF	0.05	0.03
2,3,4,7,8-PnCDF	0.5	0.3
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	0.0003

PCB	Toxic Equivalency Factor (TEFWHO98)	Toxic Equivalency Factor (TEFWHO05)
Non-ortho PCB		
PCB 77	0.0001	0.0001
PCB 81	0.0001	0.0003
PCB 126	0.1	0.1
PCB 169	0.01	0.03
Mono-ortho PCB		
PCB 105	0.0001	0.00003
PCB 114	0.0005	0.00003
PCB 118	0.0001	0.00003
PCB 123	0.0001	0.00003
PCB 156	0.0005	0.00003
PCB 157	0.0005	0.00003
PCB 167	0.00001	0.00003
PCB 189	0.0001	0.00003

List of WHO TEF (1998 AND 2005) for PCDD and PCDF.





Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Notable links

With increasing knowledge about some of the complex mixtures of POPs like PCDDs/PCDFs and PCBs it became apparent that:

- toxicological effects occurred at far lower levels with these compounds
- some individual dioxins and PCBs were far more toxicologically significant than others

These two factors put far greater demands on the analysis as they require measurement at ppt levels (as opposed to ppb and ppm) and require separation of mixtures for measurement of levels of individual compounds. Thus, more extensive concentration and cleanup are required along with greater specificity, driving methods towards employing gas chromatography/high-resolution MS (GC-HRMS) as the determination step. Magnetic sector high-resolution GC-MS has been the most established and proven technology in this field of analysis. It has been employed for more than two decades and is therefore considered to be the "gold standard" especially for the challenging analysis of dioxins and furans (PCDD/Fs).

Due to their toxicity, presence and persistence of dioxins, furans and PCBs have the most stringent regulations of all POPs worldwide. There are regulations for environmental matrices as well as food and feed material. The regulations are based on risk assessments carried out for PCBs, dioxins, and dibenzofurans at international level by the WHO (JECFA) and at the European level by EFSA. Groups of scientific experts have critically examined published toxicological studies of individual PCBs, PCDDs, or PCDFs undertaken with different animal species. The expert groups have sought to identify the level at which no adverse toxicological effect in animals is found using the most sensitive end points. This is known as the 'no observable adverse effect level' (NOAEL). Calculations are then made introducing safety factors to the NOAEL to allow for species and individual differences and then to arrive at a tolerable daily intake (TDI) or tolerable weekly intake (TWI). The TDI or TWI are toxicological levels above which

lifetime exposure is undesirable. There are inevitable slight differences in tolerable intakes proposed by different scientific bodies, depending on their data interpretation and the selected toxicological studies. In 1998, the WHO recommended a TDI of 1 to 4 pg TEQ_{WHO98} per kg body weight. For the EU, the tolerable weekly intake was set at 14 pg TEQ_{WHO98} per kg body weight, and this figure was compared with estimated exposure levels for different population groups. This exposure assessment indicated that a considerable part of the European population exceeded the TWI and therefore the European Commission developed an integrated approach to legislation on food and feed to reduce the presence of PCBs, PCDDs, and PCDFs throughout the food chain.

This approach involved:

Setting strict but feasible maximum levels in food and feed taking into account the results obtained in lowering the presence of dioxins in the environment.

Setting action levels to trigger action when levels in food or feed are found clearly above background levels. These action levels provide an early warning function.

Setting of target levels to be achieved over time so as to bring the exposure of the majority of the European population within the limits recommended by EFSA.

This approach was pursued through **Council Regulation (EC) No 2375/2001**, which established maximum levels for dioxins in meat and meat products, fish and fishery products, milk and dairy products, hen eggs and egg products, and oils and fat.



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Notable links

In the currently valid **Regulation (EC) No 1881/2006**, limits are expressed as both the sum of TEQs for PCDDs and PCDFs as well as limits for the sum of TEQs for PCDDs and PCDFs together with DL-PCBs and additionally for the sum of six PCBs (#28, #52, #101, #138, #152, and #180). The limits have been established mainly for food products from animal origin, vegetable oil, and baby food. The lowest are currently set for baby food at 0.1 pg/g WHO-PCDD/F-TEQ wet weight (0.2 pg/g WHO-PCDD/F-PCB-TEQ and 1.0 ng/g (ICES-6)) and the highest for fish products such as eel meat at 3.5 pg/g WHO-PCDD/F-TEQ wet weight (10.0 pg/g WHO-PCDD/F-PCB-TEQ and 300 ng/g (ICES-6)). Between 2006 and 2016, the Regulation has been amended 26 times, so always check the current, valid version of the regulation at www.eur-lex.europa.eu. As most human exposure to these POPs comes from animal products, it makes sense to attempt to limit these POPs in animal feed.

Directive 2002/32/EC established maximum levels for dioxins in feed materials of plant origin, minerals, binders, animal fat, and other products of animal origin, fish oil, fish meal, and compound feed, including fish feed. The regulatory limits for dioxins in animal feed range from 0.75 ng/kg WHO-PCDD/F-TEQ relative to a moisture content of 12% for all feed materials of plant origin, including vegetable oils and by-products, to 5.0 ng/kg WHO-PCDD/F-TEQ relative to a moisture content of 12% for fish oil. For dioxins together with DL-PCBs, the range is from 1.25 ng/kg to 20.0 ng/kg WHO-PCDD/F-PCB-TEQ relative to a moisture content of 12% and for the sum of six PCBs from 10 µg/kg to 170 µg/kg relative to a moisture content of 12%. All the limits are related in **Directive 2002/32/EC** including all amendments to **Regulation (EU) No 186/2015**. There are also initiatives from industry to set limits to PCDD/F and PCB concentrations in products. One of these industries is the "Global Organization for EPA and DHA Omega-3s" short GOED. Within their voluntary monograph they set limits for the sum of all 209 PCBs at 0.09mg/kg, PCDD/F at 1.75pg/g WHO-PCDD/F-TEQ and 3pg/g WHO-PCDD/F-PCB-TEQ for PCDD/F together with DL-PCBs.



Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system

Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Notable links



In addition to regulatory limits for food and feed, the European Commission has put in place strict requirements as for how sampling must be undertaken for official purposes and requirements for methods of analysis. **Regulation (EU) No 664/2017** repealing **Regulation (EU) No 589/2014** stipulates the methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs, and non-dioxin-like PCBs in certain foodstuffs. **Regulation (EU) No 771/2017** amending **Regulation (EC) No 152/2009** covers the determination of the levels of dioxins and PCBs in feeding material.

This regulation is important in enabling screening methods to be initially employed to identify potentially contaminated samples, prior to a confirmatory analysis. **'Screening methods'** are defined as methods used for selection of those samples with levels of PCDD/Fs and DL-PCBs that exceed the maximum levels or the action thresholds. The aim is to provide cost-effective, high-sample-throughput options, thus increasing the chance to discover new incidents with high exposure and health risks to consumers.

Screening methods can be bioanalytical (such as the CALUX® assay) or GC-MS methods. Results from screening indicating samples exceeding the cutoff value have to be reanalyzed by a full reanalysis from the original sample by a confirmatory method. **'Confirmatory methods'** are methods that provide full or complementary information enabling the PCDD/Fs and dioxin-like PCBs to be identified and quantified unequivocally at the maximum or, in case of need, at the action threshold. Such methods utilize gas chromatography/high-resolution mass spectrometry (GC-HRMS), which must be used with a minimum mass spectrometric resolution of 10,000 (10% valley definition). Since 2014 (regulations **(EU) No 589/2014** and **(EU) No 709/2014**), GC-MS/MS measurements are allowed as well.

The Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS and the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system offers fully compliant analysis of dioxins in food and feed in respect to EU regulations for screening methods as it meets stringent EU performance criteria. Due to its superior analytical performance, Magnetic Sector GC-HRMS is recommended for analysis of low background levels.



Thermo Scientific™ DFS™
Magnetic Sector GC-HRMS

Table of Contents

Persistent Organic Pollutants

- 1. POPs: What are POPs and why do we need to talk about them?
- 2. Introduction of classified POPs

3. Regulations and analytical methods

- PCDD/Fs and PCBs
- Non-dioxin like PCBs (NDL-PCB)
- Polybrominated diphenyl ethers (PBDEs)
- Perfluoroalkylated substances (PFOAs and PFOS)
- Pesticides
- Hexabromocyclododecane (HBCD)
- Short-chain chlorinated paraffins (SCCPs)
- Isotope dilution method
- Monitoring and surveillance of POPs

4. Future trends and recommendations

Noteable links

The analytical requirements for official methods, whether bioanalytical (only screening) or GC-MS/MS or GC-HRMS, are very detailed covering false positive/false negative rates, trueness, and precision as set out in the table below.

Analytical quality assurance measures are detailed in the regulations stipulating that adequate blanks, recoveries, and reference materials are employed to ensure the accuracy of measurements. Additionally, an EU method for feeding stuff, EN 16215:2012 "Animal feeding stuffs – Determination of dioxins and dioxin-like PCBs by GC-HRMS and of indicator PCBs by GC-HRMS" has been established.

In addition to regulatory limits, the EU has action levels that are used as a tool by competent authorities and operators to highlight cases where it is appropriate to identify a source of contamination and to take measures for its reduction or elimination.

These action limits have proven to be extremely useful particularly when monitoring the milk supply. In a number of instances, levels of PCBs and PCDD/Fs in milk from routine screening have led to the identification of sources of contamination in components of animal feed or cases of adulteration of animal feed.

Method Performance Criteria	Screening with Bioanalytical or Physico-chemical Methods	Confirmatory Methods
False compliance rate at maximum levels	<5%	—
Trueness	—	-20% – +20%
Repeatability (RSDr)	<20%	—
Within-laboratory reproducibility (RSDR)	<25%	<15%

Method performance criteria set out in Regulation (EU) NO 644/2017 and No 771/2017.

Commission Recommendation 2013/711/EU sets action levels on the reduction of the presence of dioxins, furans, and PCBs in feed and food as amended by Commission Recommendation 2014/663/EU.

Non-dioxin like PCBs (NDL-PCB)

Some countries, such as Brazil and Egypt, have also started to establish formal regulatory limits for PCDD/Fs and PCBs in food and feed. In the United States, the Food and Drug Administration (FDA) has been concerned about dioxins and has been monitoring specific foods with the goal of identifying ways to reduce dietary exposure. Since 1995, the FDA has been monitoring dioxin levels in finfish, shellfish, and dairy products, and in 1999 initiated dioxin analysis of foods collected under its Total Diet Study (TDS) survey. The TDS is an annual market basket survey covering 265 core foods (ready-to-eat) in the food supply to assess levels of various pesticide residues, contaminants, and nutrients and to estimate intakes of these substances in representative diets. Since 2002 the FDA has expanded its monitoring program to obtain more comprehensive data on background levels as well as to identify opportunities to reduce human exposure to dioxins.



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Notable links

Non-dioxin like PCBs (NDL-PCB)

In addition to regulations for food and feed matrices, several countries including the USA, Japan, and EU have established regulations to control environmental emissions of PCDD/F from incineration and other industrial processes and have put measures in place to monitor against benchmark concentrations in ambient air, environmental water, sediment, spoil, and wastewater. Methods for these regulations have been published. There are several U.S. EPA methods available for the sampling of emission samples (EPA Method 23) and for other environmental matrices like water, sludge, soil (EPA Method 8280, EPA Method 8290, and EPA Method 1613) all for PCDD/F. For PCBs, the US EPA published one method that covers all 209 PCB congeners, EPA Method 1668 "Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS." The European method for emission samples is EN 1948 "Stationary source emissions – Determination of the mass concentration of PCDDs/PCDFs and dioxin-like PCBs" parts 1 to 4. Japan established two methods for emission and water samples, JIS method K0311 "Method for determination of tetra-through octachlorodibenzo-p-dioxins, tetra-through octachlorodibenzofurans and dioxin-like polychlorinatedbiphenyls in stationary source emissions" and JIS method K0312 "Method for determination of tetra-through octachlorodibenzo-p-dioxins, tetra-through octachlorodibenzofurans and dioxin-like polychlorinatedbiphenyls in industrial water and

waste water." Many other regulations refer to the methods mentioned above, especially to US EPA Method 1613 "Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS."

Non-dioxin like PCBs (NDL-PCBs) For the non-dioxin-like PCBs (NDL-PCBs), many regulations in different countries exist. These regulations sometimes include six, seven, or even all 209 PCB congeners. The sum of the six include the PCBs 28, 52, 101, 138, 152, and 180; for the sum of the seven, PCB 118 is added to the six. For a rough estimation of the total PCB concentration of non-biological samples/matrices, the sum of the six PCBs multiplied by a factor of 4 gives a good approximation to the concentration of the total sum for all 209 congeners. Regulations for the NDL-PCBs are found for many different matrices like sewage sludge, water, indoor air, soil, etc. The sum of the six is also part of some pesticide multi-methods. The methods are usually GC methods coupled with either mass spectrometers (MS) or electron capture detectors (ECD). In some cases, like EPA Method 1668, an HRMS is required. Some examples for regulation and/or methods are California Proposition 65, the German regulation for sewage sludge (AbfKlärV), or the Brazilian Norm ABNT NBR 13882 "Líquidos isolantes elétricos – Determinação do teor de bifenilas policloradas (PCB)." ("Electrical Insulating Liquids – Determination of PCB Contents")



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Noteable links

Polybrominated diphenyl ethers (PBDEs)

EFSA conducted a risk assessment of PBDEs in 2011 that considered eight individual PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209). It was concluded that for BDE-47, BDE-153, and BDE-209, current dietary exposure in the EU did not raise a health concern. For BDE-99 there was a potential health concern with respect to dietary exposure. EFSA recommended that as numerous products containing PBDEs are still in use, surveillance of PBDEs should continue. There are currently no EU regulations concerning limits for PBDEs in food or feed, but there are restrictions on use levels. Directive 2011/65/EU on the restriction of the use of certain hazardous substances in electrical and electronic equipment include that articles may not be placed on the market if they, or flame-retarded parts thereof, contain PBDE in concentrations higher than 0.1% w/w.

A very detailed method that covers 49 of the 209 PBDE congeners is EPA Method 1614 "Brominated Diphenyl Ethers in Water Soil, Sediment and Tissue by HRGC/HRMS", but low-resolution MS (LRMS) also generates valid results for PBDEs.



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Noteable links

Fluorinated POPs are different yet again from other POPs because they are water-soluble or even exist as anions. Currently, only PFOS and its salts and their precursor perfluorooctanesulfonyl fluoride (PFOSF), precursor to PFOS-based compounds, are included in the Stockholm Convention list of POPs. Research on the distribution and transportation of the fluorinated POPs relies greatly on analysis. LC-MS/MS has been widely used for the analyses of perfluorosulfonic and perfluorocarboxylic acids including PFOS and PFOA. However, the analytical challenge remains for a direct analysis of perfluorooctanesulfonyl fluoride.



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Notable links



Chlorine-containing pesticides can be analyzed relatively easily at levels of interest by conventional extraction procedures, cleanup, and determination by GC (with ECD) or GC-MS. The analysis of pesticides including chlorinated POP pesticides is covered in a separate Thermo Scientific white paper "Analytical Challenges for Pesticide Residue Analysis in Food: Sample Preparation, Processing, Extraction and Cleanup." In addition to pesticide analysis in food, the analysis of environmental matrices may be relevant. Two example methods are EPA Method 8081 "Organochlorine pesticides by GC with ECD or ELCD" and EPA Method 1699 "Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS."



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Noteable links

Hexabromocyclododecane (HBCD)

The analysis of HBCDs is different than the OCPs, PCBs, and PCDD/PCDFs, with LC commonly used for the separation, instead of GC, in combination with a mass spectrometer, as in ISO 21677 "Determination of hexabromocyclododecane (HBCD) in water – Method using liquid chromatography-tandem mass spectrometry (LC-MS/MS)." To check building isolation material for waste disposal, an extraction with acetone and a measurement using X-ray fluorescence spectroscopy (XRF) can also identify the presence of HBCD, quickly and cheaply.





Short-chain chlorinated paraffins (SCCPs)

Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Noteable links

Due to the colossal number of individual isomers, currently, there is no method that covers all single-chlorinated paraffins. Even quantification is very difficult, as SCCPs are used as complex mixtures and are often also "contaminated" with medium-chain chlorinated paraffins (MCCP), or some shorter ones (<C10). Additionally, the analytical standard solutions available are just native technical mixtures.

One example for analytical methods is ISO 12010:2012 "Water quality – Determination of short-chain polychlorinated alkanes (SCCPs) in water – Method using gas chromatography-mass spectrometry (GC-MS) and negative-ion chemical ionization (NCI)."

For any analytical testing method, proper sampling is the first critical point on the way to valid results. As there are many specifications, sampling is also well-regulated. Sampling, transport, and even the sample container may influence the final results. For example, the analysis of phthalates in a plastic sample container will ruin the following analysis. When samples arrive at the analytical lab, sample preparation steps need to be done. In most cases, the lab will receive more than enough sample material and will be required to take a representative and homogenic part of the sample for the further analytical steps. Occasionally, it is necessary to prepare the samples for extraction. If water is present, it has to be removed or somehow bound. For extractions depending on matrix and analyte, different methods come into consideration. For dioxin analysis, it may be efficient to just dissolve the sample in solvent (*e.g.*, vegetable oil), perform a liquid/liquid extraction for water samples, perform a HCl digestion for fly ash, use a Soxhlet apparatus for dry soil samples, or an accelerated solvent extraction for feed samples like fish meal performed with Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor. For other analytes, ultrasonic, solid phase, or microwave extraction methods may be sufficient. After the extraction, a concentration step usually follows and, depending on measurement method, sometimes the extract is then ready to inject. In most cases, some type of cleanup is needed as the POPs are very persistent and most of them show no reaction if treated with sulfuric acid. A sulfuric acid coated silica gel column easily removes interferences like fat and can be used for the analysis of PBDEs. Many other chromatographic columns are possible and/or necessary depending on target analyte and target level. The lower the concentration range, the more cleanup needs to

be done to remove interferences. Other established procedures and/or reagents are gel permeation chromatography (GPC), alumina, Florisil®, carbon, and treatment with copper powder. For measurement, the perfect combination of an injector system, an analytical chromatographic column, and a detector system need to be found.

Depending on the analytes and the demand of analysis, different combinations are possible and there is not "the one system" that can handle all POPs cheaply, quickly, and with the best limits of detection. The most flexible detection system is the mass spectrometer, as it is capable of being used for all POPs.

For high sensitivity and good detection limits of different sample types in quantitative routine analysis, especially of PCDD/Fs and dioxin-like PCBs, a Magnetic Sector GC-HRMS, like the Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS should be used. Magnetic Sector technology is generally perceived as the "Gold Standard" for Dioxins and POPs analysis since it has been and still is the most prominent and established technology in dioxin expert laboratories for more than 20 years. These systems feature high robustness as needed for the wide range of sample types and a proven long instrument life time.

For the LC parameters, in POPs analysis, like PFOA, the Thermo Scientific™ Q Exactive™ Focus Hybrid quadrupole-Orbitrap™ mass spectrometer provides excellent results.

Some POPs can be completely analyzed together, like PBDE and PBB; some can share the same extraction method and the first clean-up steps, like PCB and PCDD/F.

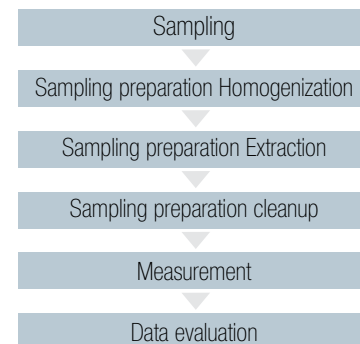


Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Notable links

Isotope dilution method

The persistence of POPs in the environment provides a strong link between transfer to foods and the need to identify sources and effectiveness of measures to reduce contamination. Thus, levels of POPs in soil, biota, water, air, and wildlife can provide early warning of build-up in the food chain and can also be used to monitor long-term trends.

International studies have concluded that approximately 95% of human exposure to POPs occurs through consumption of food of animal origin, with meat, dairy products, and fish being the main sources. Contamination of animal feed with POPs is thus an important route into the human food chain, as can be seen from many of the historical incidents involving feed particularly with dioxin and PCB contamination. While it is important to monitor food and feed for POPs and to apply action limits and regulatory controls, undertaking

risk assessment historical evidence of human exposure also provides important insights. Human biomonitoring through analysis of blood, fat, and breast milk can provide good indications of current and historical levels of exposure to organochlorine pesticide POPs, dioxins, PCBs, and corresponding brominated contaminants.

The relevance of human biomonitoring can be appreciated as a tool to follow time-trends in the figure, which shows levels of dieldrin, DDT, and PBDEs in human breast milk over 30 years. Since actions were taken to ban DDT and dieldrin, there have been sharp declines in levels in human breast milk in Sweden, a pattern which has been followed elsewhere in other countries. In contrast, over the last 10 years with increasing use of PBDEs, a sharp increase can be seen in levels in human breast milk.

Moment of addition	Name	Function
Prior sampling e.g. on absorption cartridge	Sampling standard solution	Check for errors during sampling
Prior extraction	Extraction standard solution or labelled-compound spiking solution	Used for the quantification of the results
Prior clean-up procedure	Clean-up standard solution	Check for errors during clean-up procedure
Prior injection to measurement instrument	Recovery standard solution or Internal standard solution	Determine the recovery of the whole procedure



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?

2. Introduction of classified POPs

3. Regulations and analytical methods

PCDD/Fs and PCBs

Non-dioxin like PCBs (NDL-PCB)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkylated substances (PFOAs and PFOS)

Pesticides

Hexabromocyclododecane (HBCD)

Short-chain chlorinated paraffins (SCCPs)

Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Notable links

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Table of Contents

Persistent Organic Pollutants

- 1. POPs: What are POPs and why do we need to talk about them?
- 2. Introduction of classified POPs

3. Regulations and analytical methods

- PCDD/Fs and PCBs
- Non-dioxin like PCBs (NDL-PCB)
- Polybrominated diphenyl ethers (PBDEs)
- Perfluoroalkylated substances (PFOAs and PFOS)
- Pesticides
- Hexabromocyclododecane (HBCD)
- Short-chain chlorinated paraffins (SCCPs)
- Isotope dilution method

Monitoring and surveillance of POPs

4. Future trends and recommendations

Notable links



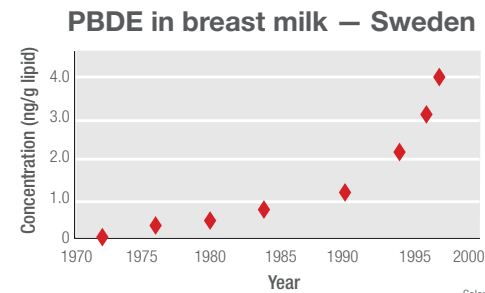
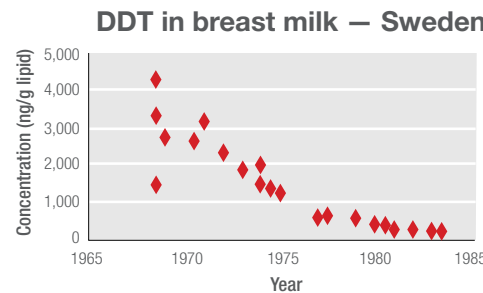
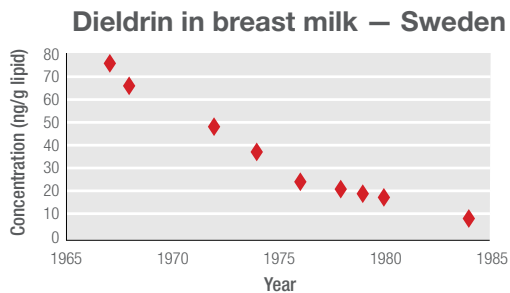
Monitoring and surveillance of POPs

Previously, there were a rather limited number of laboratories that had GC-HRMS and had invested in the extensive cleanup facilities necessary to undertake PCDD/PCDF analysis. Although many laboratories are primarily focused on food testing, it was not unusual for the range of matrices analyzed in these laboratories to extend to include environmental as well as human biomonitoring samples. A common scenario would be to identify a dioxin "hot-spot" perhaps through monitoring cow's milk, and then analyze feed, herbage, and soil to pinpoint contamination sources.

This would be commonly followed by analysis of blood samples from exposed population groups to look for evidence of accumulated POPs through long-term exposure, compared to normal background levels.

It is not possible however to analyze environmental samples, like fly-ash from incinerators, in the same laboratories undertaking food or biomonitoring samples, as cross-contamination from samples containing these high levels is very difficult to control, resulting in high laboratory background levels evident in blank samples run as controls.

Example of the Efficacy of Interventions POPs in Breast Milk



Solomon 2002



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs
3. Regulations and analytical methods
4. Future trends and recommendations

Notable links

The list of chemicals currently being considered by the Stockholm Convention is only a small fraction of the approximately 30,000 chemicals in worldwide commercial use, many of which are potential POPs. Criteria for new POP candidates in terms of their persistence, long-range transport, bioaccumulation, and toxicity are fulfilled by many halogenated organics, cyclic siloxanes, and substituted aromatics. Many laboratories world-wide seeking to identify emerging contaminants are focusing on environmental samples, and recent publications indicate that the Thermo Scientific™ Orbitrap™ mass analyzer technology is playing a major role in identifying unknowns. These can be not only the primary contaminants, but also their transformation products.

Even when PCDDs/PCDFs and DL-PCBs need to stand apart due to the demands of sensitivity and specificity for other application areas an expansion of the scope of analytical methods for emerging and potential POPs is foreseen, where compatible classes of POPs are monitored in multi-analyte methods. The Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap™ GC-MS/MS system features the combination of high-resolution gas chromatography and high-resolution, accurate-mass (HRAM) Orbitrap mass spectrometry. It is a dedicated benchtop GC-MS system and powerful tool for emerging POPs research.

For Magnetic Sector GC-HRMS, the most prominent and established technology for quantitative routine analysis of dioxins and POPs, development continues as well. The DFS Magnetic Sector GC-HRMS with the DualData XL Module allows increasing the sample throughput up to a factor of 2. This productivity increase is achieved by staggered injection of samples on two GC systems of the DFS Magnetic Sector GC-HRMS. Both GC systems are working simultaneously, synchronized by a dedicated software and hardware. This new development enables laboratories to run drastically more samples per given period of time on a single GC-HRMS, deliver results faster and lower their price-per-sample costs.

[Thermo Scientific™ Exactive™
GC Orbitrap™ GC-MS system](#)



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs
3. Regulations and analytical methods
4. Future trends and recommendations

Notable links

There is a need to look for previously unknown organofluorine compounds in environmental samples such as water and sewage that have led to the development of non-targeted analytical methods. LC-Orbitrap MS is playing a particularly significant role in discovery and characterization of novel PFASs in environmental water samples. In some methods, industrial water samples are centrifuged and the supernatant directly injected, while in other methods various SPE cartridges are used for extraction and concentration of contaminants. In a recent publication, novel polyfluorinated sulfates, chlorine-substituted perfluorocarboxylates, and hydro-substituted perfluorocarboxylates were discovered in industrial water using Orbitrap mass analyzer technology. Complementary and starting at the opposite end of the food chain is biomonitoring of human samples such as blood and urine to look for evidence of human exposure to PFASs, and again Orbitrap mass analyzer technology is playing a major role in identification of novel fluorinated compounds. Perfluorinated compounds have been commonly monitored in food by LC-MS/MS. However, Orbitrap mass analyzers offer an attractive alternative for these halogenated chemicals due to their significant mass defect. Particularly in fish samples, there can be matrix effects caused by co-eluting and isobaric interferences that can be overcome by using HRMS. The high stability of perfluorinated compounds leads to a relatively poor and non-specific fragmentation pathway in MS/MS. In addition, biliary acid-interfering compounds in fish can cause interferences in LC-MS/MS. In contrast, HRMS on a Thermo Scientific™ LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap Mass Spectrometer system in full-scan mode offers both sensitivity and specificity due to the typical mass defect characterizing these halogenated compounds. The higher specificity also circumvents the risk of overestimation of levels.

Future trends and recommendations



Table of Contents

Persistent Organic Pollutants

1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs
3. Regulations and analytical methods
4. Future trends and recommendations

Noteable links

Future trends and recommendations

For the future, more profiling of xenobiotics in human matrices in an untargeted mode will be pursued to provide insights into human exposure to POPs and other contaminants.

The development and applications of HRMS have shown potential and promise to greatly expand the ability to capture the broad spectrum of environmental chemicals in human exposure studies. HRMS can perform both untargeted and targeted analysis, because of its capability of full- and/or tandem-mass spectrum acquisition at high mass accuracy with good sensitivity. The collected data from target, suspect, and non-target screening can be used not only for the prospective identification of environmental chemical contaminants in human matrices but also retrospectively.

The 'bread and butter' work using Magnetic Sector GC-HRMS, GC-MS/MS and GC-MS to monitor the well-established POPs will inevitably continue in order to protect human health via food safety control and control of POPs exposure into the environment.

However, for the future, the two-pronged approach of untargeted analysis of environmental samples as potential exposure sources of POPs and human biomonitoring as evidence of actual exposure will provide useful tools to set priorities. Unquestionably the use of Orbitrap mass analyzer will play an increasingly significant role in environmental analysis and human biomonitoring as well as meet the demands of analysis of newly identified POPs in foods.



1. POPs: What are POPs and why do we need to talk about them?
2. Introduction of classified POPs
3. Regulations and analytical methods
4. Future trends and recommendations

EU Regulations, Directives and Recommendations:

- a) REGULATION (EU) 2017/644: <http://data.europa.eu/eli/reg/2017/644/oj>
- b) REGULATION (EU) 2017/771: <http://data.europa.eu/eli/reg/2017/771/oj>
- c) DIRECTIVE 2011/65/EU: <http://data.europa.eu/eli/dir/2011/65/oj>
- d) RECOMMENDATION 2013/711/EU: <http://data.europa.eu/eli/reco/2013/711/oj>
- e) DIRECTIVE 2002/32/EC: <http://data.europa.eu/eli/dir/2002/32/oj>
- f) REGULATION (EU) 2015/186: <http://data.europa.eu/eli/reg/2015/186/oj>
- g) REGULATION (EC) No 1881/2006: <http://data.europa.eu/eli/reg/2006/1881/oj>
- h) REGULATION (EC) NO 396/2005: <http://data.europa.eu/eli/reg/2005/396/oj>

Selection of methods:

- a) US EPA 1614: <https://www.epa.gov>
- b) US EPA 1699: <https://www.epa.gov>
- c) US EPA 23: <https://www.epa.gov>
- d) US EPA 8280: <https://www.epa.gov>
- e) US EPA 8290: <https://www.epa.gov>
- f) US EPA 1613: <https://www.epa.gov>
- g) US EPA 1668: <https://www.epa.gov>
- h) US EPA 8081: <https://www.epa.gov>
- i) JIS K0311: <http://www.jsa.or.jp/>
- j) JIS K0312: <http://www.jsa.or.jp/>
- k) EN 1948: <https://standards.cen.eu/>
- l) EN 16215: <https://standards.cen.eu/>
- m) ISO 21677: <https://www.iso.org/standards.html>
- n) ISO 12010: <https://www.iso.org/standards.html>





Table of Contents

Persistent Organic Pollutants

- 1. POPs: What are POPs and why do we need to talk about them?
- 2. Introduction of classified POPs
- 3. Regulations and analytical methods
- 4. Future trends and recommendations

Noteable links

Other documents:

- a) GOED monograph: <http://goedomega3.com/index.php/goed-monograph>
- b) SANTE/11945/2015: <http://www.crl-pesticides.eu>

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