VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ
BRNO UNIVERSITY OF TECHNOLOGY

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DISERTAČNÍ PRÁCE
PhD THESIS

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PERSISTENTNÍ ORGANICKÉ POLUTANTY V ŽIVOTNÍM PROSTŘEDÍ JIHOMORAVSKÉHO KRAJE

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PhD THESIS

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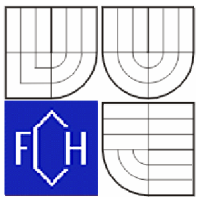

PhD thesis (October 2008)

Persistent organic pollutants in the environment of the Southern Moravia region

The Institute of Chemistry and Technology of Environmental Protection
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SUMMARY

Persistent organic pollutants such as polychlorinated biphenyls (PCBs) or organochlorine pesticides (OCPs) are ubiquitous environmental contaminants. Due to their physico-chemical properties they tend to persist in the environment for a long time and accumulate in adipose tissues of wildlife and humans. Despite many international efforts and measures to ban or strongly limit their use, their levels in the global environment seem to decrease only slowly.

Bioaccumulation of POPs in an aquatic food web and historical trends in POPs recorded in sediment layers were studied in the Brno Lake's environment. Current levels of organochlorines were determined in the chub from the Svratka River and the hygienic quality of fishes from recreational fishing was evaluated as well. None of these surveys showed higher contamination and the present levels of POPs were comparable with other similar findings. Analyses of the sediment cores showed moderate decreasing trends in POPs.

Current levels of POPs in terrestrial environment were determined using conifer needles as a suitable biomonitoring tool. Needles from different localities in the Czech Republic were sampled during 2007 and investigated for the content of common organochlorine pollutants. The obtained results showed relatively low levels of POPs and the contamination determined in the needles was supposed to originate mainly from the long-range transport via atmosphere. Compared with some similar studies from the CR and with findings from other countries, the data on POPs in the needles from CR seemed to be of little importance and just above the limits of detection in many cases.

Specimens of three raptor species were collected at localities of Central Moravia during 2003-2007 and investigated for the content of PCBs and organochlorine pesticides. In addition to that, POPs were determined in sediment and fish prey of water raptors from the Záhlice Ponds in order to document the process of bioaccumulation of persistent pollutants in the food chain. The findings were comparable with those from some foreign studies despite the fact there were significant intraspecies differences and the concentrations of POPs did not follow normal distribution. Generally, the determined levels of the contaminants seemed not to be of greater importance (except few outlying values). Further, significant differences were found in the distribution of POPs among individual tissues of the cormorant and bird feathers showed to be a good tool for monitoring the contamination of (at least) cormorants with POPs as the levels determined in muscle and feathers were in good correlation.

The last part of this thesis presents the results of an assessment of two modern extraction techniques – Pressurised Solvent Extraction (PSE) and Microwave-assisted Extraction (MAE) – for the determination of chlorinated POPs in both abiotic and biotic samples. Both PSE and MAE methods offered accurate date with a good precision. Moreover, these techniques were found to be quite easy to operate and low solvent and time consuming, contrary to conventional techniques such as Soxhlet extraction or sonication.

Key words:
Polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), bioaccumulation, aquatic ecosystem, fish, raptors, needles, contamination

SOUHRN

Persistentní organické polutanty (POP) jako polychlorované bifenyly (PCB) nebo organochlorované pesticidy (OCP) jsou všudypřítomné kontaminanty životního prostředí. Fyzikálně-chemické vlastnosti těchto látek způsobují mj. jejich rezistenci vůči rozkladu a hromadění v tukových tkáních živočichů včetně lidí. I přes četné mezinárodní snahy a opatření směřující k jejich zákazu či značnému omezení použití jejich hladiny v životním prostředí klesají jen pozvolna.

Jedním z cílů této práce bylo posouzení bioakumulace POP v potravním řetězci Brněnské přehrady a sledování časových změn koncentrací POP v jejích sedimentárních vrstvách. Dále byly hodnoceny hladiny chlorovaných polutantů v jelci tloušti ze dvou lokalit na řece Svratce pod Brnem a hygienická kvalita ryb ulovených při amatérském rybolovu na různých nádržích. Výsledky neprokázaly, že by se jednalo o zvýšené koncentrace, které byly srovnatelné s podobně zaměřenými studiemi. Vyšetřované ryby splnily limity dané příslušnými vyhláškami a analýzy sedimentárních vrtů ukázaly zvolna klesající trendy koncentrací POP.

Současný stav kontaminace terestrického prostředí byl zhodnocen pomocí stanovení POP ve vzorcích jehličí, které bylo odebráno na různých místech ČR v průběhu roku 2007. Naměřená data ukázala relativně nízké hladiny POP v jehličí a tato kontaminace byla přisouzena zejména dálkovému přenosu těchto látek atmosférou. Zjištěné koncentrace patřily k těm nižším ve srovnání s jinými státy a v mnoha případech se pohybovaly na hranici detekčních limitů.

Kontaminace dravých ptáků organochlorovanými polutanty byla posuzována na jedincích tří druhů, kteří pocházeli z oblasti Střední Moravy v letech 2003 až 2007. Dále byla hodnocena kumulace POP v rámci vodního potravního řetězce v Záhlických rybnících u Kroměříže. Výsledky studie byly srovnatelné se zahraničními nálezy, navzdory skutečnosti, že byly pozorovány značné rozdíly i mezi jedinci stejného druhu. Hladiny POP nebyly obecně závažné (až na několik odlehlých hodnot). Dále byly zjištěny rozdíly v distribuci sledovaných kontaminantů v různých tkáních kormorána a byla potvrzena využitelnost per pro nedestruktivní stanovení hladin kontaminantů u dravých ptáků.

Poslední část této disertace přináší výsledky posuzování dvou moderních extrakčních technik – tlakové extrakce rozpouštědlem (PSE) a mikrovlnné extrakce (MAE) – určených pro stanovení POP v různých matricích. Obě techniky poskytovaly dobré výtěžnosti a opakovatelnosti, jejich obsluha byla jednoduchá a vykazovaly nižší spotřebu rozpouštědel oproti klasickým technikám jako Soxhlet nebo ultrazvuk. Navíc měly výrazně kratší extrakční dobu.

Klíčová slova:
Polychlorované bifenyly (PCB), organochlorované pesticidy (OCP), bioakumulace, vodní ekosystém, ryby, dravci, jehličí, kontaminace

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PROHLÁŠENÍ

Prohlašuji, že jsem disertační práci vypracoval samostatně a že všechny použité literární zdroje jsem správně a úplně citoval. Disertační práce je z hlediska obsahu majetkem Fakulty chemické VUT v Brně a může být využita ke komerčním účelům jen se souhlasem vedoucího disertační práce a děkana FCH VUT.

.....
podpis doktoranda

Poděkování

Na tomto místě bych chtěl poděkovat všem, kteří mi jakýmkoliv způsobem pomohli předkládanou práci dovést do konce.

Velké poděkování patří zejména vedoucí této disertace paní prof. RNDr. Miladě Vávrové, CSc., za odborné vedení, cenné rady a pomoc při zajišťování výzkumného materiálů. Rovněž bych chtěl poděkovat pracovníkům Laboratoře organických polutantů Ústavu veterinární ekologie a ochrany životního prostředí na VFU v Brně za pomoc při zpracovávání vzorků, jmenovitě pak paní Alžbětě Kábrtové a Ireně Malotové. Nesmím rovněž opomenout kolegyně a kolegy z Ústavu chemie a technologie ochrany životního prostředí FCH VUT v čele s vedoucím ústavu doc. Ing. Josefem Čáslavským, CSc. za jejich pomoc a rady.

V neposlední řadě si velké díky a uznání zaslouží Míša, má rodina a všichni blízcí za jejich trpělivost a podporu během vypracovávání této práce.

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1. INTRODUCTION

A rapid development of human society during the past two centuries, particularly in relation to the scientific and industrial revolution, entailed not only huge positive changes in the living standard of most people (especially in developed countries), but has also brought considerable negative impacts on the environment as a penalty for this progress. New discoveries and technologies allow resolving many problems; on the other hand, they brought about brand-new, often even more serious troubles.

It is estimated that there are between one and two million preparations on the market today as well as countless manufactured articles made from chemicals. Chemical industry makes up an important part of the world economy. With an estimated US$1500 milliard in sales in 1998 [**1**], the chemical industry accounts for 9% of international trade [**2**]. The annual production of organic compounds reached the level of half a milliard tonnes in 1990, and the volume of the world output doubles in every seven or eight years. According to the *OSN Programme of the Environment*, people have already discovered or developed more than 7 million chemicals – and there are thousands of new substances arising every year [**3**].

Certain chemicals released to the air, water or soil can have direct or indirect impacts on human health and the environment. In many cases, we have to face a dirty heritage of the past, when new compounds having excellent properties had been produced and used wholesale, but later they were identified as a serious threat. Exposure to certain hazardous substances can lead to a direct toxic effect on human health and/or the environment. Unlike e.g. climate changing substances, which have been largely identified, the nature and magnitude of hazardous substances released during chemical production processes and use are far from known. Nonetheless, using the data that are available, governments have developed lists of substances considered to pose risks to human health and/or the environment and that require national control. However, a very limited number of hazardous substances are included in internationally agreed lists at present.

A problem of one group of chemicals having specific properties dangerous to the environment was brought to the awareness of the public in 1962, when the book 'Silent Spring' written by Rachel Carson was published. Author's effort to point out increasing threats of organochlorine insecticides as aldrin, DDT, dieldrin, lindan etc., which had been widely used since the Second World War, started a wave of publications and scientific activities. These early activities concerned what we now call POPs – persistent organic pollutants – and resulted in adopting international regulations over these compounds. Starting in the early 1970s, one country after another restricted or banned the use of POP pesticides, often with the use of DDT for public health applications (disease vector control) as the only exemption. On the basis of new scientific findings, new compounds like polychlorinated biphenyls, polycyclic aromatic hydrocarbons or dioxins were gradually classed as the POPs. To that end, two international legally binding instruments have been negotiated and concluded.

The first one was The Protocol to the regional UNECE Convention on Long-Range Transboundary Air Pollution (CLRTAP) on POPs, opened for signatures in June 1998 and entered into force on 23 October 2003, the objective of which is to control, reduce or eliminate discharges, emissions and losses of POPs [**4**]. The CLRTAP POP protocol regulates 16 compounds or mixtures of compounds at different levels, and defines how further compounds can be added to the existing list of persistent compounds. According to this protocol, POPs are defined as organic substances that possess toxic characteristics in a broad

sense, are persistent, bioaccumulate, tend to long-range transboundary atmospheric transport and deposition, and are likely to cause significant adverse human health or environmental effects near to and distant from their sources.

Unlike the LRTAP POP protocol, which covers the UN ECE region, another international treaty, The Stockholm Convention on persistent organic pollutants from May 2001, is accepted globally and has specific targets even in developing countries [**5**]. The convention binds its Parties to the elimination of production and use, or to the limited use of selected substances including organochlorine pesticides (OCPs), industrial chemicals like polychlorinated biphenyls (PCBs) or hexachlorobenzene (HCB) and undesired by-products (polychlorinated dibenzo-*p*-dioxins – PCDDs). In addition to banning the use of POPs, the treaty focuses on cleaning up the growing accumulation of unwanted and obsolete stockpiles of pesticides and toxic chemicals that contain POPs. Having been ratified by 50 countries, the SC on POPs entered into force on 17 May 2004 and became international law.

Despite these broad international efforts to prevent POPs from leaking into the environment and to remove old burdens, the levels of POPs being found in both abiotic and biotic parts of the nature decrease only slowly and POPs continue to accumulate through the food chain leading up to humans. Another problem is the fact that we have only little information on the synergistic effects of a number of various POPs present in an organism together, or on their possible interactions with other chemical substances at present. And these substances are most often, in real environments, present in the form of complex mixtures.

Main objective of this study is to bring new information on the levels of selected POPs in the environment and on the analytical approach to POPs following previous and present studies, and to outline subsequent research in this field. Close attention should be paid especially to the bioaccumulation of chlorinated compounds in the food chains and to evaluating the efficiencies of new extraction methods which could be used as more suitable tools replacing common extraction techniques.

2. POPS - LITERATURE REVIEW

2.1. Properties & Sources of POPs

As stated in **Chapter 1**, persistent organic pollutants (POPs) or persistent, bioaccumulative and toxic compounds (PBTs) are mainly synthetics chemical compounds that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to the human health and the environment. This group of priority pollutants consists of pesticides (such as DDT), industrial chemicals (such as polychlorinated biphenyls - PCBs) and unintentional by-products of industrial processes (such as polychlorinated dibenzodioxins and furans). POPs are transported across international boundaries far from their sources, even to regions where they have never been used or produced. The ecosystems and indigenous people of the Arctic are particularly at risk because of the long-range environmental transportation and biomagnification of these substances. Consequently, persistent organic pollutants pose a threat to the environment and to human health all over the globe.

The following chapters deal with 'the state of the art' of three main members of the POP family: polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs). The rest of POPs (PBTs) will be briefly mentioned as well.

2.1.1. Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are a large group of synthetic aromatic compounds which do not occur naturally in the environment. They consist of the biphenyl structure with two linked benzene rings in which some or all of the hydrogen atoms are substituted by chlorine atoms (basic molecular structure in **Fig. 1**).

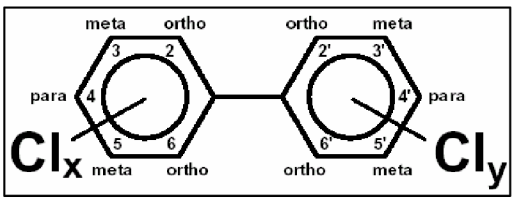


Fig. 1: Molecular structure of PCBs

PCBs are manufactured commercially by a progressive chlorination of biphenyl in the presence of a suitable catalyst (e.g., iron chloride). Depending on the reaction conditions, the degree of chlorination can vary between 21 and 68% (w/w). The yield is always a mixture of different isomers and congeners. Thus, a total of 209 theoretically different chemical components can exist, but only about 130 congeners are likely to occur in commercial products or mixtures of such compounds [**6**].

The chemical formula of PCBs is $C_{12}H_{10-n}Cl_n$, where n ranges from 1 to 10. Since the identification of individual PCBs based on their systematic names showed to be inconvenient, Ballschmiter and Zell [7] proposed a simple numbering system for the PCB congeners which has been adopted by the International Union of Pure and Applied Chemists (IUPAC). The overview of all 209 PCB congeners is shown in **Fig. 2**.

The terminology of PCBs includes three terms: *homologue, isomer* and *congener*. *Homologues* vary in the number of chlorine atoms and there are ten PCB homologues (monochloro- to decachlorobiphenyl). PCB *isomers* vary in the positions of chlorine atoms. And finally, PCB *congener* means a specific compound having the exact number and defined positions of the chlorine atoms.

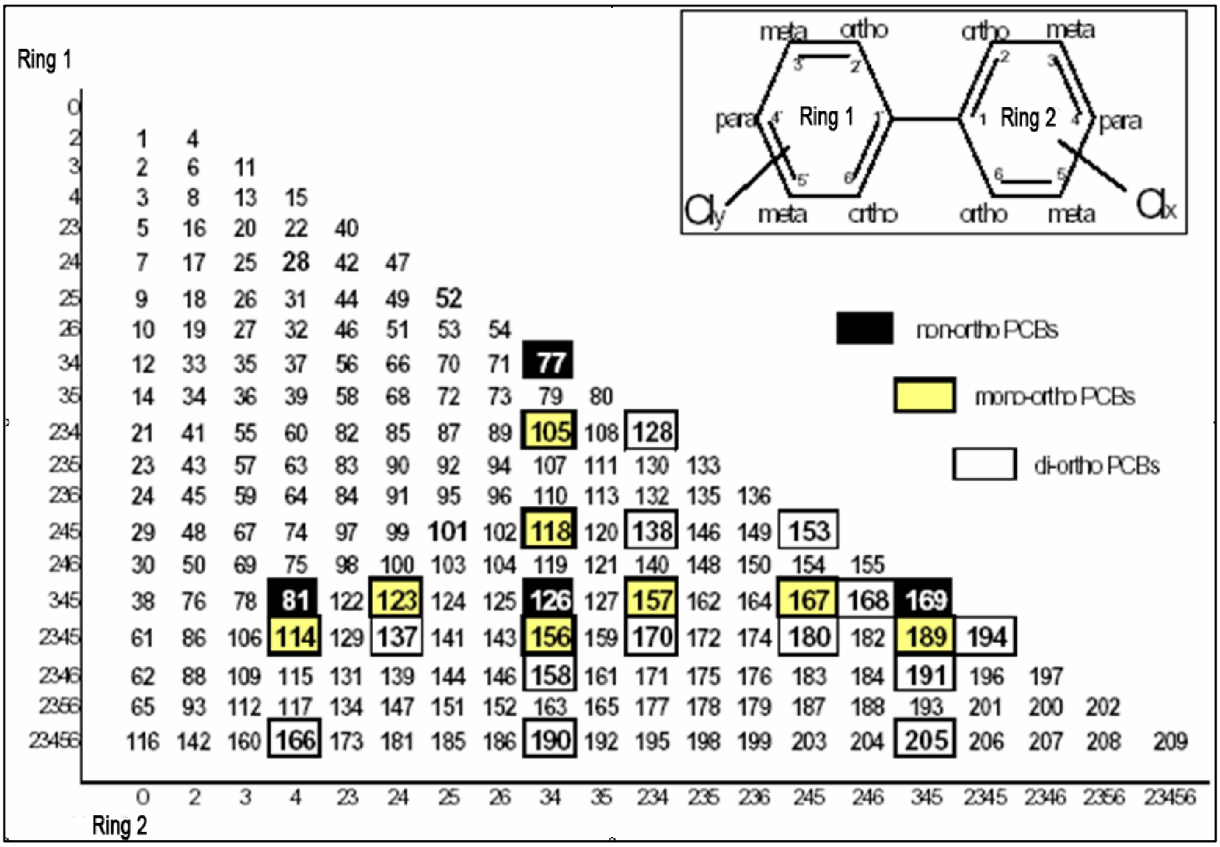


Fig. 2: Systematic nomenclature of PCB congeners [7]; Important congeners highlighted.

Tab. 1: Physical and chemical properties of indicator PCB congeners [8]

PCB congener **Systematic name**	**CAS index**	**M** [g/mol]	**b. p.** [°C]	**water solubility** [mg/L] (25 °C)	**log** K_{OW}	**H*)** (25 °C)
PCB 28 2,4,4'-trichlorobiphenyl	7012-37-5	257.2	340	0.067-0.266	5.8	31.6
PCB 52 2,2',5,5'-tetrachlorobiphenyl	35693-99-3	292.0	360	0.006-0.074	6.1	47.6
PCB 101 2,2',4,5,5'-pentachlorobiphenyl	37680-73-2	326.4	380	0.0042-0.031	6.4	35.5
PCB 118 2,3',4,4',5-pentachlorobiphenyl	31508-00-6	326.4	380	0,002[a]	6.4	40.5
PCB 138 2,2',3,4,4',5'-hexachlorobiphenyl	35065-28-2	360.9	400	0.0009-0.01	7.0	48.6
PCB 153 2,2',4,4',5,5'-hexachlorobiphenyl	35065-27-1	360.9	400	0.001	6.9	42.9
PCB 180 2,2',3,4,4',5,5'-heptachlorobiphenyl	35065-29-1	395.3	400[a]	0.00063[a]	7.4	-

*) [Pa m^3 mol^{-1}], [a] approx.

Seventy-eight out of the possible 209 PCB congeners can exist as rotational isomers that are enantiomeric to each other. And it is widely recognized that certain biological activities of PCBs vary, at least quantitatively, with stereochemical differences in the congeners.

Individual pure PCB congeners are colourless, often crystalline compounds, but the commercial PCBs are mixtures of these congeners with a clear, light yellow or dark colour. They do not crystallise at low temperatures, but turn into solid resins. Because of the chlorine atoms in the molecule, their density is rather high. PCBs are, in practice, fire resistant with rather high flash-points (170-380 °C), form vapours heavier than the air, but do not form any explosive mixtures with it, and possess very low electrical conductivity and an extremely high resistance to the thermal breakdown. Some physical and chemical data for a number of indicator congeners are presented in **Tables 1** and **3**.

Industrial production of PCBs

PCBs were synthesised for the first time in 1881 and their usefulness for industry, due to their physical properties, was recognised early. PCBs have been used commercially since their first production in the USA in 1929 as dielectric and heat-exchange fluids and in a variety of other applications.

By far the most important producer of PCBs was *Monsanto Chemical Co.* (USA) and its technical PCB mixtures were named *Aroclor® 12xx*; biphenyls are generally indicated by 12 in the first 2 positions, while the last 2 numbers indicate the weight percentage of chlorine in the mixture. For example, Aroclor 1260 should contain approximately 60 wt.% of chlorine. Two notable exceptions to this numbering system are Aroclor 1016, which is a technical mixture derived by distillation of Aroclor 1242, and Aroclor 1232 which is a blend of approximately equal proportions of Aroclors 1221 and 1242 [**10**]. Twelve different Aroclors containing from 21 to 68 wt.% of chlorine were produced and, due to their excellent technological properties, the PCB production soon expanded and other world companies put their 'PCBs' on the market (**Table 2**). According to some sources, the estimated cumulative production of PCBs could near 1.5 million tons [**9**].

Tab. 2: Total world PCB production [9]

Producer	Country	Period	Amount (t)	Some trade names
Monsato, Geneva Ind.	USA	1930 - 1977	641 700	Aceclor, Aroclor,
Kanegafuchi, Mitsubishi	Japan	1954 - 1972	58 787	Arubren, Biclor,
Bayer AG	West Germany	1930 - 1983	159 062	Blacol, Clophen,
Prodelec	France	1930 - 1984	134 654	Delor, Delotherm,
S.A. Cros	Spain	1955 - 1984	29 012	Dykanol, Hydelor,
Monsato	UK	1954 - 1977	66 542	Kanechlor, PCBs,
Caffaro	Italy	1958 - 1983	31 092	Phenoclor,
Chemko	Czechoslovakia	1959 - 1984	21 482	Pyralene, Pyranol,
Orgsteklo, Orgsintez	USSR (Russia)	1939 - 1993	173 800	Pyroclor,
Xi'an	China	1960 - 1979	8 000	Santotherm,
Total		1930 - 1993	**1 324 131 t**	Sovol, Sovtol, …

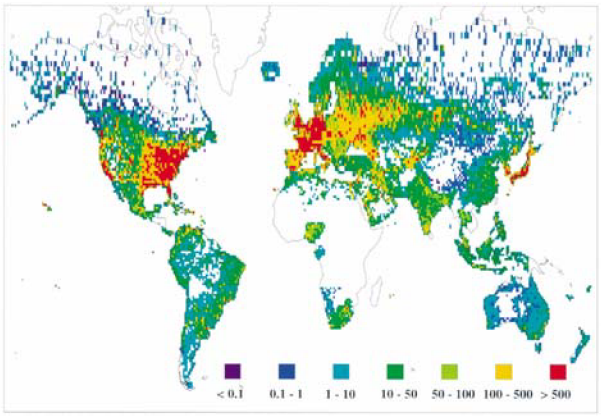


Fig. 3: Estim. global usage of PCBs (in tons) [**9**]

Fig. 3 suggests that almost 97% of the intentionally produced PCBs have been used in Northern Hemisphere; and their utilisation was really universal. The reasons for such a broad range of using PCBs were particularly the simplicity and economy of the PCB production, their unique properties and low acute toxicity of PCBs.

Unfortunately, these 'unique' properties also made PCBs highly resistant to any natural biodegradation and during the 1960s, PCBs began to be found in various samples from the environment [**12**]. The climax – the production of 33 000 tons per year – came in 1970 and then the production decreased, was significantly limited and gradually completely terminated [**11**].

Tab. 3: (I) Important physical and chemical properties of PCBs and (II) their applications [**11**]

✓ fire resistant (flash-points 170-380°C) ✓ high degree of chemical stability ✓ very low electrical conductivity ✓ extremely high resistance to thermal breakdown ✓ practically insoluble in water; easily in hydrocarbons, fats, and other organic compounds ✓ do not form any explosive mixtures with air ✓ excellent thermal conductivity	**Closed systems** transformers, small and large capacitors, hydraulic and heat transfer fluids, PCB-containing fire-resistant hydraulic fluids in coal mining equipment, lubricants **Open systems** plasticisers, carbonless copy paper, lubricating oils, inks, laminating and impregnating agents, paints, adhesives, waxes, additives in cement and plaster, casting agents, dedusting agents, sealing liquids, fire retardants, immersion oils and pesticide extenders, carbonless copy paper

In the former Czechoslovakia, however, where PCB production began in 1959, a completely different development took place. Ignoring alarming and generally available information regarding the danger of PCBs, in 1972, their production started to rise and reached its peak around 1980 (terminated in 1984). Technical mixtures of PCBs were manufactured in *Chemko Strážské n.p.* (Eastern Slovakia) as *Delor 103, 106* (similar to Aroclors 1242 and 1260), *Hydelor* and *Delotherm*. More than 21.5 million kilograms of PCBs were produced and half of this amount was exported. It is believed that 31% of PCBs have leaked into the environment, 65% are still used in closed systems, and only 4% have been disposed. The PCB products from *Chemko* were used by more than 200 different organisations in the Czechoslovakia, and thus compiling complete database is nearly impossible [**13**]. Hard to believe that some PCB containing products remained on the market until 1993, despite the ban on production and known fact.

Toxicology of PCBs

The acute toxicity of PCBs, after a single oral exposure, is generally low and thus they had been considered to be 'biologically inactive' until the early 1970s. The LD_{50} values vary among species, particularly due to wide divergences in biological effects, mainly in the order of units of grams per kg body weight, and depend on the PCB congener contents. There are many various effects observed in animals after exposition to PCBs. It is known that some species like rats or fish are less resistant to low chlorinated biphenyls, unlike another species (e.g. birds) which are more sensitive to acute doses of more chlorinated PCB congeners. As for mammals, minks are considered to be the most sensitive. There is a clear evidence that PCBs cause cancer in animals, but, on the other hand, there are many contradictory results of studies concerning PCBs and their cancer risks to humans. The WHO International Agency for Research on Cancer (IARC) has declared PCBs to be probably carcinogenic to humans (2A category) [**16**] as well as the National Toxicology Program (USA) which has stated that it is reasonable to conclude PCBs are carcinogenic in humans.

At present, acute PCB intoxication in humans is almost impossible, however, two large episodes of intoxication in humans occurred in the past.

Tab 4: The 'Yusho' a 'Yu-Cheng' intoxication symptoms [**14**]

Effect on:	**Anamnesis:**
skin	hyperkeratosis, hyperpigmentation, chloracne
glands	swelling, hypersecretion
nails, mucous membranes	pigmentation
liver	elevated levels of serum alkaline phosphatase, decreased serum bilirubin levels
lungs	chronic bronchitis (due to methylsulfone metabolites)
serum lipids	elevated serum triglyceride levels
immune system	increases in the occurrence of infections, decreases in serum immunoglobulin levels (IgA and IgM), ↓ cellular immunity
female reproduction system	affected menstrual cycle
nervous system	decrease in the conduction velocity in peripheral sensory nerves, peripheral sensory neuropathy
placenta	enzyme activity induction
newborns	↓ birth weight, skin and mucous membranes pigmentation, facial oedema, abnormal calcification of the skull

In 1968, a large number of people in Japan were accidentally poisoned by the consumption of a batch of rice oil contaminated with Kanechlor 400. The average estimated intake was 633 mg PCBs, 3.4 mg PCDFs, and 596 mg PCQs, roughly equivalent to 157 µg PCBs/kg per day, 0.9 µg PCDFs/kg per day, and 148 µg PCQs/kg body weight per day [**18,19**]. A similar accident happened in the Province of Taiwan in 1979, where the affected people had also consumed rice-bran oil contaminated with PCBs. The two cases of poisoning were called Yusho and Yu-Cheng accidents, respectively. The main symptoms in Yusho and Yu-Cheng patients have frequently been attributed to contaminants in the PCB mixtures, specifically, to PCDFs and PCDDs, which were found in both contaminated food samples and attacked people [**17,18**]. The patients suffered from severe chloracne and various troubles. The overview of all main symptoms and signs of Yusho and Yu-Cheng is summarised in **Table 4**.

Metabolism of PCBs

The biodegradation of PCBs occurs either in sediments (anaerobic dechlorination by bacteria) or in animal bodies by metabolic transformations. PCBs are able to induce various enzymes involved in degradation of xenobiotics. Detoxification system is located in the liver cells and called 'mixed function oxygenase' (oxidase and oxygenase function, MFO). The process of biotransformation begins by an incorporation of one oxygen atom into xenobiotics and by parallel oxidation of NADPH; in the following stage the conjugation with endogenous metabolites (glutathione, glucuronic acid, sulphate) takes place and these pathways allow organism to excrete the xenobiotics easily [**20**]. The system includes pyridine dehydrogenase, flavoproteine, ferroproteine and cytochrome P-450 which reacts with oxygen and forms unstable complex transferring oxygen to the xenobiotics. Like other biotransformation enzymes, the monooxygenase system also shows inductive effects, i.e., the synthesis increases rapidly by a chronic exposure to a certain compound (e.g. PCBs), which results in its hydroxylation and easier elimination of more polar metabolites [**21**].

The initial step in the biotransformation of PCBs involves CYP enzyme (cytochrome P-450) (CYP1A1, 1A2, and CYP2B1/2B2) mediated oxidation of arene oxides, which are mainly transformed to hydroxylated aromatic compounds but also to sulphur-containing metabolites via the mercapturic acid pathway [**23**]. Unsubstituted *meta* and *para* carbon atoms are the preferred site for oxidation [**24**], hydroxylation of coplanar PCBs usually predominates at the *para* position in the least chlorinated phenyl ring, and the rate of metabolism generally decreases with increasing chlorine substitution [**25**]. Most hydroxylated PCB metabolites are excreted in faeces and/or in urine, or conjugated to glucuronic acid or sulphate, however, several hydroxylated PCB metabolites are retained in the body, either due to their high lipophilicity or reversible binding to proteins which can initiate undesirable cell interferences. Although several of the OH-PCBs are present in rat plasma and seal blood, the spectrum of OH-PCBs is different in human plasma, compared to that in rats or seals [**29**]. In general, the persistent OH-PCBs have chlorine atoms on the adjacent carbons to the OH-group and contain five or more chlorine atoms. Methylsulfonyl ($MeSO_2$) metabolites of PCBs have been widely detected in the tissues of marine mammals and of humans [**26, 27**], and are e.g. possibly etiologically connected to the respiratory toxicity described in Yusho victims [**28**]. Since certain hydroxylated and methylsulfonyl PCB metabolites are present at levels higher than their respective parent compounds in some cases, it is necessary to investigate further potential biological and/or toxicological activities of these persistent metabolites. The summary of the structures of PCB metabolites is presented in the **Figure 4**.

Some biological activities of PCBs involve initial Ah-receptor mediated mechanisms (e.g., induction of hepatic CYP1A oxygenases and Phase II enzymes such as UDP glucuronyl transferases, epoxide hydrolases, or glutathione transferase, body wasting, thymic atrophy, and porphyria), other activities involve Ah-receptor independent mechanisms (e.g., induction of CYP2B and CYP3A oxygenases, induction of changes in brain dopamine levels, and disruption of Ca^{2+} homeostasis), and other biological activities of PCBs may involve both Ah-receptor dependent and independent mechanisms (e.g., liver hypertrophy, disruption of steroid hormone homeostasis or thyroid hormone homeostasis, disruption of immune functions, and induction and promotion of liver cancer). Because of this diversity in biological activities, there is a large potential for opportunities for PCB mixtures to alter the toxicity of other chemicals or other chemicals to alter the toxicity of PCBs.

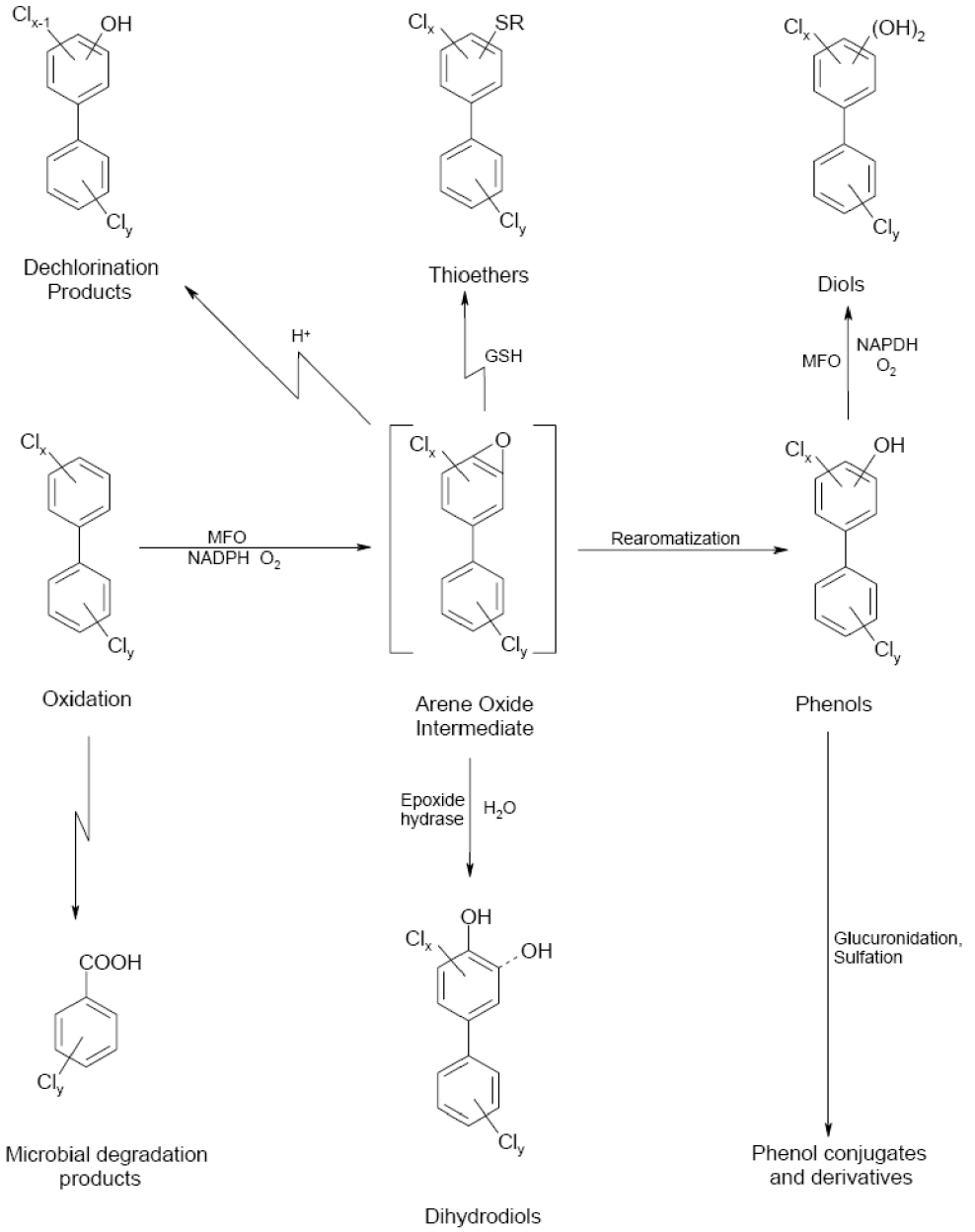


Fig. 4: Metabolic pathways for polychlorinated biphenyls [**22**]

PCB mixtures display different induction profiles of mixed-function oxidase (MFO), so that the individual PCB congeners can be phenobarbital-type (PB) (~50 congeners), 3-methylcholanthrene-type (3-MC), or mixed-type inducers, or they may be inactive as enzyme inducers (most congeners). PB induces *a*, *b* and *c* isoenzymes of cytochrome P-450 (CYP $2B_1$ and $2B_2$) stimulating metabolism of a broad range of compounds and increasing Ah cytosol receptor level. 3-MC induces hepatic aryl hydrocarbon hydroxylase (AHH) and ethoxyresofurin- O-deethylase (EROD). As a result of this induction type, immunotoxicity, teratogenicity, dermal alterations, liver disorders or reproductive toxicity occur [**30**]. The effects of commercial PCB mixtures are called mixed induction (PB/3-MC type), because of the induction via different isoenzymes of both groups. The 36 PCB congeners, considered environmentally threatening due to their frequency of occurrence in environmental samples, abundance in technical mixtures and potential toxicity, are listed in **Table 5** [**31**].

Tab. 5: Selected PCB congeners according to their toxicity and abundance; (**indicator congeners**)

High toxicity and very abundant[1]	Less toxicity but very abundant[2]	High abundance[3]	Potentially toxic but low presence[4]
3 MC Type Inducers	**PB Type Inducers**	**Non Inducers Type**	**Mixed Type Inducers**
77: 3,3',4,4'-TeCB	87: 2,2',3,4,5'-PeCB	18: 2,2',5-TrCB	37: 3,4,4'-TrCB
126: 3,3',4,4',5-PeCB	99: 2,2',4,4',5-PeCB	44: 2,2',3,5'-TeCB	81: 3,4,4',5-TeCB
169: 3,3',4,4',5,5'-HxCB	**101: 2,2',4,5,5'-PeCB**	49: 2,2',4,5'-TeCB	114: 2,3,4,4',5-PeCB
	153: 2,2',4,4',5,5'-HxCB	**52: 2,2',5,5'-TeCB**	119: 2,3',4,4',6-PeCB
Mix Type Inducers	**180: 2,2',3,4,4',5,5'-HpCB**	70: 2,3',4',5-TeCB	123: 2',3,4,4',5-PeCB
105: 2,3,3',4,4'-PeCB	183: 2,2',3,4,4',5',6-HpCB	74: 2,4,4',5-TeCB	157: 2,3,3',4,4',5'-HxCB
118: 2,3',4,4',5-PeCB	194: 2,2',3,3',4,4',5,5'-OcCB	151: 2,2',3,5,5',6-HxCB	158: 2,3,3',4,4',6-HxCB
128: 2,2',3,3',4,4'-HxCB		177: 2,2',3,3',4',5,6-HpCB	167: 2,3',4,4',5,5'-HxCB
138: 2,2',3,4,4',5'-HxCB		187: 2,2',3,4',5,5',6-HpCB	168: 2,3',4,4',5',6-HxCB
156: 2,3,3',4,4',5'-HxCB		201: 2,2',3,3',4,5,5',6'-OcCB	189: 2,3,3',4,4',5,5'-HpCB
170: 2,2',3,3',4,4',5-HpCB			

[1] 3-MC type inducers and mix type inducers very abundant in the environment
[2] PB type inducers are less toxic but most abundant in the environment
[3] Weak or non-inducers representing about 10 % of the PCB content of tissues
[4] Some potential for toxicity but very low presence in tissues

Toxic (planar) PCB congeners and toxicity assessment

It is generally accepted that those PCB congeners (i.e. those that can assume the planar configuration and exhibit a high affinity for the Ah receptor) are more potent toxicants than the other congeners (i.e., those with multiple chlorine substitution in ring positions 2 and 6) [**36**]. The planar (or coplanar) PCB congeners from the group of the non- and mono *ortho*-substituted PCBs have one chlorine in the *meta* position and one or no chlorine in the *ortho* position. Recent researches have shown that some di- and mono-*ortho* analogues of the non-*ortho* planar PCBs may possess comparable toxic potential thanks to their ability to induce the 3-MC type isoenzymes [**37**]. The analogous structure-activity relations of these PCB congeners with respect to most of their toxic responses indicate that they are approximate stereoisomers of 2,3,7,8,-tetra-chlorodibenzo-*p*-dioxin (TCDD). The mechanism is based on the binding affinity of dioxin-like PCB congeners to the cytosol Ah-receptor protein, a product of the regulator Ah gene. The induction is dependent on the position and number of chlorine atoms in the molecule and the congeners that bind most strongly to the Ah-receptor show the strongest induction of monooxygenases and the highest toxicity. The Ah-receptor can be found in many animal species, including humans [**38**]. The activated ligand-receptor complex translocates into the nucleus and binds to specific regions on the DNA chain, designated as dioxin-responsive elements (DREs). Binding to the DREs activates nearby genes including the gene for cytochrome P450 $1A_1$. Activation leads to the increased rates of transcription of mRNA which undergo translation leading to production of the proteins coded for, among them the cytochrome P450 $1A_1$. Altered levels of these proteins have secondary and tertiary effects on a cell function, which are manifested as many toxic responses to TCDD and other dioxin-like substances. For example, the induction of the cytochrome P450 $1A_1$ leads to an increased production of hepatic enzymes such as AHH and EROD [**39**]. Binding to the Ah receptor is not enough to elicit a biological response. The mechanism of action of dioxin-like substances seems to be similar to that found for steroid hormones. The steps in this process are shown in **Figure 5** [**33**].

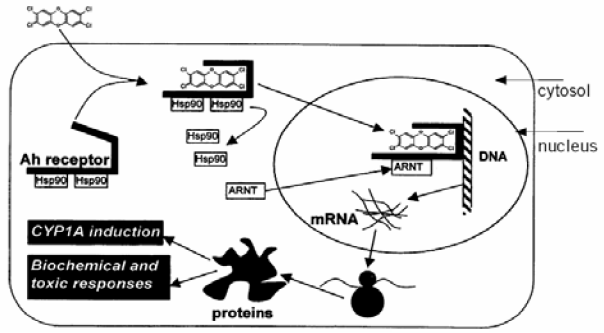


Fig. 5: The proposed mechanism of action of PCDDs/PCDFs and related substances.

As the other PCDD, PCDF and PCB congeners produce similar effects but are less potent than TCDD, various schemes have been developed to assess the risk of mixtures of those compounds. In these toxic equivalency models, each dioxin-like compound is given a toxic equivalency factor (TEF) based on its toxicity relative to that of 2,3,7,8-TCDD using *in vitro* and *in vivo* data. These include the Ah receptor binding affinity, enzyme induction, cancer, reproductive toxicity etc. 2,3,7,8-TCDD, the most toxic congener, is given a TEF of 1. Each congener's concentration is multiplied by its TEF to give the relative TCDD toxicity of that congener in the mixture, its so-called TCDD equivalency (TEQ). The TEQs for all congeners can be calculated and added together to give the total TCDD toxicity in the mixture. The TCDD toxic equivalency model, however, has its limitations owing to a number of simplifications. The most important limitation is that the combined toxic effects of the components of a given mixture are assumed to be additive, neglecting possible synergism or antagonism. Furthermore, differences in the toxicokinetics of individual congeners are not always taken into account.

$$\mathbf{TEQ = \Sigma\,[(PCDD_i)_{conc.} \times TEF_i] + \Sigma\,[(PCDF_i)_{conc.} \times TEF_i] + \Sigma\,[(PCB_i)_{conc.} \times TEF_i]}$$

The following criteria for including a compound in a TEF-scheme should be met:

- ✓ it should show a structural relationship to the PCDDs and PCDFs
- ✓ it should bind to the Ah-receptor
- ✓ it should elicit dioxin-specific biochemical and toxic responses
- ✓ it should be persistent and accumulative in the food chain.

On the basis of these criteria and after evaluating the present database, the WHO European Centre for Environment and Health and the International Programme on Chemical Safety (IPCS) have recommended interim TEFs for four non-*ortho*-, eight mono-*ortho*- and two di-*ortho*-substituted PCBs listed in **Table 6**.

The major route for human exposure to dioxin-like substances is via food. Several different approaches have been used by different countries in order to establish tolerable daily intake (TDI) values for TCDD. The US Environmental Protection Agency (EPA) has opted for the most conservative model using a linear multistage extrapolation model based on carcinogenicity in rats. TCDD is assumed to be an ultimate carcinogen, both initiating and promoting tumour growth. This model assumes a linear relationship between dose and tumour incidence even with doses approaching zero. Using this approach, EPA has calculated a TDI of 0.006 pg TCDD/kg body weight. Most other countries have assumed that TCDD is a promotor only, with a threshold for its effects. The no-observable effect level (NOEL) of 1000 pg/kg body weight/day has been used. This level is then divided by a safety factor to derive a final TDI. E.g. for the Nordic countries, the safety factor of 200 has been used leading to a recommended TDI of 5 pg TCDD/kg body weight/day [**40**]. Other countries using this model have TDIs of 1-10 pg TCDD/kg body weight/day.

Using essentially the same data, but also recognising the toxicokinetic differences between humans and rodents, in 1990, the WHO recommended a TDI of 10 pg TCDD/kg body weight/day [**42**]. In the new review of recent research on low dose effects on the immune system, and development of the reproductive and neurobehavioral systems (for examples see [**41**]), in 1998, WHO has revised their TDI to 1-4 pg TCDD/kg body weight/day [**43**]. In 2000, the EU Scientific Committee on Food (SCF) has recommended an interim tolerable weekly intake (TWI) of 7 pg WHO-TEQ/kg body weight [**44**].

Tab. 6: TEF schemes for selected PCBs, PCDDs and PCDFs – previous and *current values

PCB congener	**Safe 1990 [32]**	**Ahlborg 1992**	**Safe 1994 [33]**	**Ahlborg 1994 [34]**	**Van den Berg (WHO I-TEF)* 1998 [35]**		
					Human/mammals	Fish	Birds
77	0.01	0.0005	0.01	0.0005	0.0001	0.0001	0.05
81	-	-	-	-	0.0001	0.0005	0.1
126	0.1	0.1	0.1	0.1	0.1	0.005	0.1
169	0.05	0.01	0.05	0.01	0.01	0.00005	0.001
105	0.001	0.0001	0.001	0.0001	0.0001	<0.000005	0.0001
114	0.001	0.0005	0.0002	0.0005	0.0005	<0.000005	0.0001
118	0.001	0.0001	0.0001	0.0001	0.0001	<0.000005	0.00001
123	0.001	0.0001	0.00005	0.0001	0.0001	<0.000005	0.00001
156	0.001	0.001	0.0004	0.0005	0.0005	<0.000005	0.0001
157	0.001	0.001	0.0003	0.0005	0.0005	<0.000005	0.0001
167	0.001	-	-	0.00001	0.00001	<0.000005	0.00001
170	-	-	-	0.0001	-	-	-
180	-	-	-	0.0001	-	-	-
189	0.001	-	-	0.00001	0.0001	<0.000005	0.00001
2,3,7,8-TCDD	1	1	1	1	1	1	1
1,2,3,7,8-PCDD	0.5	0.5	0.5		1	1	1
2,3,7,8-TCDF	0.1	0.1	0.1		0.1	0.05	1

For the average consumer, a daily intake of dioxin-like PCBs determined as TEQs would be 10–30% of the TDI. When the contribution from the PCDDs and PCDFs is taken into account, the intake would increase to 20–60%, i.e. 100-600 pg TEQ/person/day. There are, however, groups with specific dietary habits (e.g. a high intake of contaminated food) or occupational exposure that may exceed the TDI for PCDDs and PCDFs.

Environmental concentrations of PCDDs/PCDFs and coplanar PCBs have declined continuously since the 1970s and this indicates that the current mean intake of dioxin-like compounds is probably somewhat lower than that in 1990.

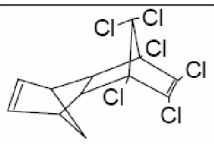
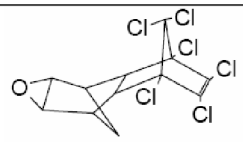
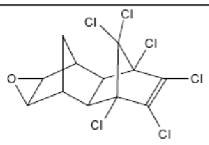
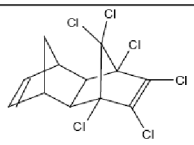
2.1.2. Organochlorine pesticides (OCPs)

In general, pesticides are substances used to prevent, destroy, repel or mitigate any pest ranging from insects, animals and weeds to micro-organisms such as fungi, moulds, bacteria and viruses. Prior to about 1940, pesticides were primarily inorganic chemicals or natural agents from plant origin (e.g. nicotine). From approx. 1940 to 1980, however, an exponential increase in the production and use of synthetic pesticides such as DDT and other mainly chlorinated hydrocarbons was evident worldwide. While the public health and economic benefits of synthetic pesticide are indisputable, the findings of widespread environmental contamination by OCPs, reaching global proportions, heralded the end of an era for their extensive use and OCPs have been removed from the market due to their adverse health and environmental effects and their persistence.

Nowadays most OCPs are included e.g. in the Stockholm Convention on POPs (May 2001) and are banned from production and use, or strictly limited in use, and their occurrence in the environment including humans is intensively and systematically monitored. The following chapters describe basic properties as well as the history (not only) of the 'Stockholm Convention' OCPs and other organochlorine compounds.

Polychlorinated cyclodienes (aldrin, dieldrin, endrin and isodrin)

This group of pesticides is derived from cyclodiens. Aldrin (and its stereoisomer isodrin) was first synthesised by the Diels-Alder condensation in the USA in 1948, dieldrin (and its stereoisomer endrin) was manufactured by the epoxidation of aldrin. Technical-grade aldrin contains not less than 85.5% of aldrin (trade names include Aldrec, Aldrex, Drinox, Octalene, Seedrin etc.), technical-grade dieldrin contains not less than 85% of dieldrin (Alvit, Dieldrix, Octalox, Quintox, and Red).

				
(CAS)	**aldrin** (309-00-2)	**dieldrin** (60-57-1)	**endrin** (72-20-8)	**isordin** (465-73-6)
P/U[+]	None/Restricted	None/Restricted	None/None	-
log K_{OW}	6.50	6.20	5.60	-
sol. (25)*	0.18 mg/L	0.20 mg/L	0.25 mg/L	-

[+] production/use nowadays (accord. to SC POPs 2001); * solubility in water at 25 °C

Aldrin and dieldrin were popular pesticides for corn and cotton crops, were used as a prophylactic and for treatment of timber against termite infestation. Nowadays they are no longer produced worldwide and their use is restricted (e.g., permitted in India till depleted). In the former Czechoslovakia, aldrin was used against soil-dwelling pests (reg. 1962-1963, ban in 1980), dieldrin was used for seed treatment (reg. 1962-1968) and endrin used against field mice (reg. 1960-1983, ban in 1984). However, less information on total amounts and uses is available [**46**].

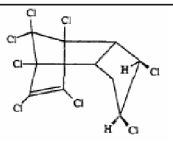
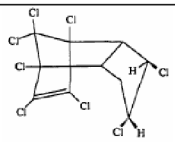
Chemical and biological decomposition of polychlorinated cyclodienes takes place in a number of dechlorinating, dehydrochlorinating and hydroxylating reactions. In biological systems of soils, plants, and animals, aldrin converts rapidly to dieldrin by a microsomal oxidation reaction (epoxidation) and degrades very slowly (the half-life in temperate soils is

about 5 years, while it disappears more quickly (up to 90% in 1 month) from tropical soils). Soil residues of dieldrin are found in higher concentration and with greater frequency than residues of aldrin, even though aldrin was applied more frequently to the soil [**47**]. Several metabolites become relatively soluble in water. They undergo photolytic changes and degradation when exposed to light.

As for the toxicity, central nervous system excitation is the primary adverse effect observed in humans in cases of the aldrin or dieldrin intoxication. Aldrin and dieldrin are carcinogenic in animals, but this effect appears to be specific to the mouse liver. According to the IARC, aldrin, dieldrin and endrin are not classifiable as to carcinogenicity to humans (cat. 3) [**45**]. Based on studies in animals, the EPA has determined that aldrin and dieldrin are probable human carcinogens. No information was located regarding effects on the endocrine system in humans following oral exposure. On the other hand, an adverse effect of exposure to sufficiently high levels of aldrin or dieldrin on male fertility cannot be excluded.

Chlordane

Chlordane is a mixture of more than 100 related chemicals, of which about 10 are major components such as *cis*- and *trans*-chlordane (60-85%) or heptachlor. Chlordane has been used worldwide as a pesticide on agricultural crops, lawns, and gardens, as a fumigating agent and to control termites in homes. Nowadays its production/using is restricted and allowed only in few countries. Chlordane was never registered, produced or used in the Czech Republic [**46**].

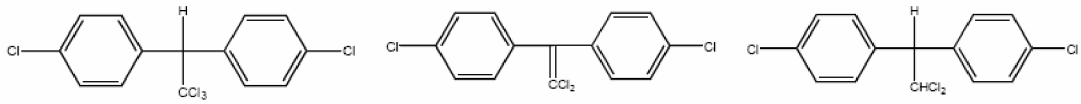
		
	α-chlordane (*cis*)	**γ-chlordane** (*trans*)
(CAS)	(5103-71-9)	(5103-74-2)
P/U[+]	Restricted/Restricted	
log K_{OW}	5.54 (estim. for pure chlordane)	
sol. (25)*	0.056 mg/L (for cis:trans 75:25)	

On the basis of historic production figures, an estimated 70,000 tons of chlordane were produced since 1946, 25-50% of which still exists unaltered in the environment. This material was exclusively released to soil [**48**]. Chlordane may be transported to long distances in the atmosphere. In soil, chlordane adsorbs to the organic matter and volatilises slowly over time. In general, it remains in the top 20 cm of most soils, and in some soils it stays at this level for over 20 years [**49**].

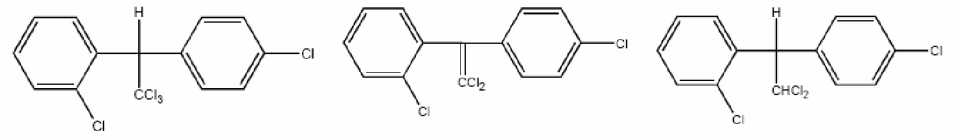
Only a few microorganisms have been isolated that are capable of degrading chlordane. Chlordane metabolism results in a number of oxidation products, including oxy-chlordane, which persist in body fat as the predominant chlordane residues. As for its toxicity, most health effects in humans linked to the chlordane exposure are on the nervous system, the digestive system, and the liver. According to the IARC chlordane is possibly carcinogenic to humans (cat. 2B, 2001) [**45**]. Chlordane is highly toxic for aquatic animals, esp. for shellfish and fish.

DDT and its metabolites (DDTs)

DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) is a pesticide that has been used to control insects on agricultural crops and insects that carry diseases like malaria and typhus, but now it is used in only a few countries to control malaria. Technical-grade DDT is a mixture of three forms, *p,p'*-DDT (85%), *o,p'*-DDT (15%), and *o,o'*-DDT (trace amounts).



(CAS)	***p,p'*-DDT** (50-29-3)	***p,p'*-DDE** (72-55-9)	***p,p'*-DDD** (72-54-8)
log K_{OW}	6.20	5.76	6.02
sol.(25)*	0.025 mg/L	0.12 mg/L	0.090 mg/L
P/U^{+}		Restricted/Restricted	



(CAS)	***o,p'*-DDT** (789-02-6)	***o,p'*-DDE** (3424-82-6)	***o,p'*-DDD** (53-19-0)

[+] production/use nowadays (accord. to SC POPs 2001); * solubility in water at 25 °C

Technical grade DDT may also contain DDE and DDD as contaminants. DDD was also used to kill pests, but to a far lesser extent than DDT. One form of DDD (*o,p'*-DDD) has been used medically to treat cancer of the adrenal gland. DDT does not occur naturally in the environment. DDT, DDE, and DDD last in the soil for a very long time, potentially for tens of years. Most DDT breaks down slowly into highly stable DDE and DDD, generally by the action of microorganisms (see **Figure 6**).

During the 1970s, the use of DDT was terminated in the United States and in Europe, except in cases of a public health emergency. It is, however, still produced and used in some other parts of the world, most notably for controlling malaria. As for the CR, DDT was banned for use as the pesticide in 1974, although even after this year DDT was used in limited quantities in selected products, for example for head lice liquidation in e.g. Neratidine product; it was phased out between 1978-1983 [**46**].

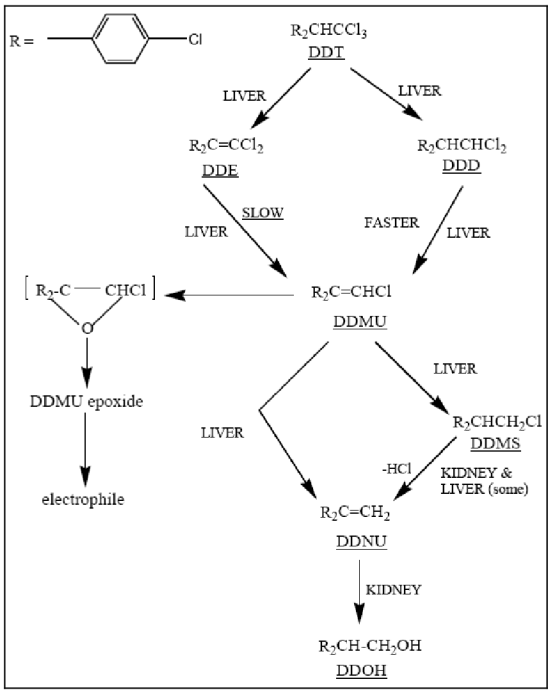
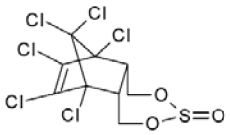
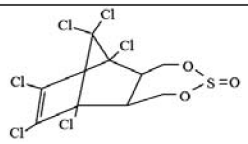
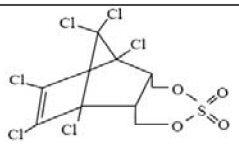


Fig. 6: Metabolic scheme for DDT [**50**]

DDT and its metabolites are highly toxic for fish and birds [**51**]. As for humans, the general population is currently exposed to DDT and its metabolites primarily in food and there are measurable quantities in many commodities. DDT belongs to "*endocrine disrupting chemicals*" which may affect the hormonal homeostasis and effects could be seen on several systems including the reproductive system. The *p,p'*-DDT isomer is possibly carcinogenic to humans (cat. 2B, IARC 2001) [**45**].

Endosulfan (I and II)

Endosulfan is structurally similar to other polychlorinated cyclodienes (aldrin, dieldrin or chlordane). It has been used to control a number of insects on food crops such as grains, tea, fruits, and vegetables and on non-food crops such as tobacco and cotton.

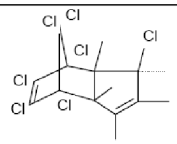
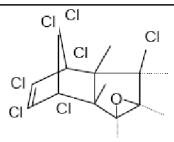
			
(CAS)	***α*-endosulfan** (959-98-8)	***β*-endosulfan** (33213-65-9)	**endos. sulphate** (1031-07-8)
P/U[+]	Produced/Used	Produced/Used	-
log K_{OW}	4.94	4.78	-
sol. (25)*	0.53 mg/L	0.28 mg/L	-

[+] production/use nowadays; * solubility in water at 25 °C

Technical endosulfan is comprised of 70% *α*-endosulfan and 30% *β*-endosulfan, with the *α*-isomer being more toxic than the *β*-isomer. The oxidative degradation product of endosulfan, endosulfan sulfate, was found to be more toxic than the parent compounds [**54**]. In the Czech Republic, its use is authorised until stock depletion (Thiodan 35EC; currant, strawberry, or flowers; 8 kg in 2001) [**53**]. Endosulfan is more toxic than DDT, is very toxic to fish and other aquatic organisms [**52**], and belongs to "*endocrine disrupting chemicals*". IARC and EPA have not classified endosulfan as to its ability to cause cancer.

Heptachlor

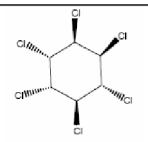
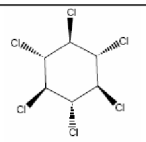
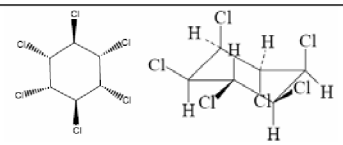
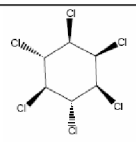
Heptachlor is chemically related to endosulfan and was mainly used in the 1960s and 1970s to control termites and ants. It is both a breakdown product and a component of the pesticide chlordane (approximately 10% by weight). Its insecticidal effects were described at the beginning of the 1950's, after its isolation from the technical chlordane. It was commercially produced by Velsicol Chemical Corp. above all. Nowadays (after SC 2001) it is used in only a few countries (Russia, Algeria, etc.). It is not produced in the Czech Republic, and its use for agricultural purposes was banned in 1989. Heptachlor epoxide is an oxidation product of heptachlor, which is formed in bacteria, plants and animals. Heptachlor epoxide breaks down very slowly in the environment and it can stay in soils and water for many years. Both heptachlor and heptachlor epoxide build up in fish and in cattle. The available data suggest that the developing nervous system is the most sensitive target of heptachlor toxicity [**55**]. Experiments on mice and rats proved certain carcinogenic effects of heptachlor. According to the IARC as well as to the EPA, heptachlor is possibly carcinogenic to humans (cat. 2B, 2001) [**45**].

		
(CAS)	**heptachlor** (76-44-8)	**hept. epoxide** (1024-57-3)
P/U[+]	None/Restricted	-
log K_{OW}	5.94	5.42
sol. (25)*	0.056 mg/L	0.28 mg/L

[+] production/use nowadays; * solubility in water at 25 °C

Hexachlorocyclohexanes (HCHs)

HCH consists of eight isomers [**56**]. The γ-HCH is considered to be the most efficient insecticide out of five stereoisomers formed by the chlorination of benzene. The pesticide lindane refers to the products that contain >99% of γ-HCH, and is produced and used as an insecticide on fruit, vegetables, and forest crops, and animals and animal premises.

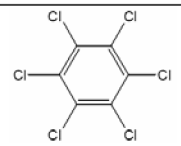
				
	α-HCH	**β-HCH**	**γ-HCH (lindane)**	**δ-HCH**
(CAS)	(319-84-6)	(319-85-7)	(58-89-9)	(319-86-8)
log K_{OW}	3.80	3.78	3.55	4.14
sol. (25)*	10 mg/L	7 mg/L	17 mg/L	9 mg/L

[+] production/use nowadays; * solubility in water at 25 °C

Technical-grade HCH typically contain about 10–15% of γ-HCH as well as the α-, β-, δ-, and ε- isomers which are commonly detected in the environment. The β-HCH is the most persistent isomer, due to the equatorial positions of chlorines. In the former Czechoslovakia, in total 3,330 tons of lindane were produced, that is about 5% of the production of technical HCH. Lindane was used in combination with DDT (preparations Lydikol and Gamadyn); after the DDT ban, it was still used for the seed treatment. Currently, its use in agriculture is not permitted [**46**]. In animals, γ-HCH appears to be transformed by hepatic enzymes to form chlorophenols, chlorobenzenes, chlorocyclohexanes, etc. HCHs affect the nervous and reproductive systems, and show hepatotoxicity. All HCH isomers are possibly carcinogenic to humans (cat. 2B) [**45**].

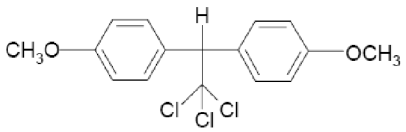
Hexachlorobenzene (HCB)

is an ubiquitous environmental pollutant which was once used as a fungicide on grains, however, its major source is now thought to be as a production by-product of a large number of chlorinated compounds, particularly lower chlorinated benzenes, and pesticides [**57**]. HCB is not produced in the Czech Republic; its production was terminated in Spolana Neratovice in 1968. Thus, HCB is a by-product of the tetrachloromethane, perchloroethylene, trichloroethylene or pentachlorobenzene productions. HCB is also formed by the electrolysis – production of the chlorine, together with octachlorostyrene [**46**]. HCB tends to remain in the environment for a long time (a half-life of 3–6 years in soils). HCB is a major airborne organochlorine in the arctic air present at relatively uniform picograms-per-cubic-metre concentrations [**58**]. HCB is slowly metabolised to chlorophenols by the cytochrome P-450 system, or reductively dechlorinated to form pentachlorobenzene. HCB also belongs to "*dioxin-like*" toxicity compounds, with the TEF value (related to TCDD) of 0.0001 (like PCB 105, 118, 189, etc.), and it has e.g. teratogenic and neuropathologic effects. HCB is possibly carcinogenic to humans (cat. 2B, IARC 2001) [**45**].

	
(CAS)	**HCB** (118-74-1)
P/U[+]	Restricted/Restricted
log K_{OW}	5.73
sol. (25)*	0.006 mg/L

Methoxychlor

is a manufactured chemical still used for controlling insects. It is effective against flies, mosquitoes, and a wide variety of other insects. Since methoxychlor is more unstable than DDT, it has less residual effect. It has been used extensively e.g. in Canada for a control of biting flies. In the USA, MXC is listed as a PBT chemical (EPA), and the technical products of methoxychlor was suspended in 2000 and all tolerances were cancelled in 2002. In the former Czechloslovakia, methoxychlor was produced by Spolana Neratovice from 1965 to 1972 in relatively small quantities due to a low interest. Methoxychlor (MXC) is moderately persistent in soils (half-time of ~120 days in aerobic soils). It is slightly toxic to bird species, but highly toxic to fish and aquatic invertebrates. Methoxychlor can accumulate in some living organisms, including algae, bacteria, snails, clams, and some fish. However, most fish and animals change MXC into other substances that are rapidly released from their bodies, so MXC does not usually build up in the food chain [**59**]. It has the potential to cause damage to liver, kidney and heart tissue. There is no evidence of carcinogenicity in humans [**45**]. MXC belongs to endocrine disrupting chemicals which may affect hormonal homeostasis and effects could be seen on several systems including the reproductive system [**60**].

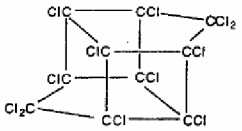


(CAS)	**methoxychlor** (72-43-5)
P/U[+]	Restricted/Restricted
log K_{OW}	5.08
sol. (25)*	0.045 mg/L

[+] production/use nowadays; [*] solubility at 25 °C

Mirex

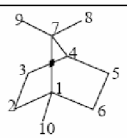
Mirex is an insecticide used for controlling fire ants. Its production/use was banned (excluding Australia and China; SC 2001). In the USA, mirex was most commonly used in the 1960s and 1970s, and all uses were cancelled in 1978. It has never been produced, registered or used in the Czech Republic. Mirex degrades to photomirex, which is even more poisonous. It has harmful effects on stomach and intestines, damages the liver, kidneys and the nervous system and the reproductive system. Mirex has been listed as a PBT pollutant target by EPA and is possibly carcinogenic to humans (cat. 2B, IARC 1987) [**45**].



(CAS)	**mirex** (2385-85-5)
P/U[+]	Restricted/Restricted
log K_{OW}	5.28
sol. (25)*	0.6 mg/L

Toxaphene

Toxaphene is an insecticide containing over 670 polychlorinated bicyclic terpenes consisting predominantly of chlorinated camphenes. It is not produced in the Czech Republic and its use was banned in 1986. In the years 1963 – 1987, a large quantity of products containing toxaphen (Melipax) was imported into the former Czechoslovakia [**46**]. It injures the kidneys and liver, damages the immune system, arms the adrenal gland and causes changes in the development of unborn children. Toxaphene is possibly carcinogenic to humans (cat. 2B, IARC 2001) [**45**].

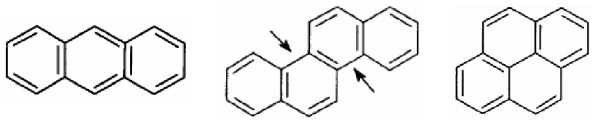


1-10 = Cl

(CAS)	**toxaphene** (8001-35-2)
P/U[+]	None/None
log K_{OW}	
sol. (25)*	

2.1.3. Polycyclic aromatic hydrocarbons (PAHs)

PAHs are a large group of organic compounds produced by both human activities and natural processes. They are formed by at least two condensed benzene rings in a linear, angular, or cluster arrangement (such a structure is also referred to as a fused ring system; see **Figure 7**), and furthermore they can be substituted in many ways which results in an immense diversity of forms. PAHs generally occur as complex mixtures, not as single compounds. When referring to these compounds, various terms including *polycyclic aromatic compounds* (PACs), *polynuclear aromatics* (PNAs) or *polycyclic organic matter* (POM) are often used as well. PAHs include hundreds of compounds which have attracted much attention because many of them are carcinogenic, especially those PAHs containing four to six aromatic rings.

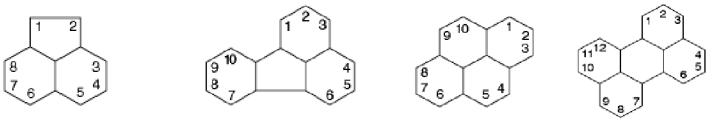


linear (anthracene) **angular** (chrysene) **cluster** (pyrene)

Fig. 7: Structural arrangement of PAHs

The nomenclature is not fully uniform in the literature, despite the fact that the IUPAC has adopted a nomenclature which is commonly used nowadays [**61, 62**]. The IUPAC names are based on the largest parent PAH with a retained trivial name (examples in **Figure 8**).

When a choice exists, the fragment lowest in the IUPAC list (i.e., with the largest number) should be used. The simplest attachments are then selected for naming. Structures are typically oriented such that (1) the greatest number of rings in a row are aligned horizontally, (2) the maximum number of rings are positioned in the upper right quadrant, and (3) the least number of rings are positioned in the lower left quadrant. The numbering begins with the uppermost ring the furthest to the right, with the most counter-clockwise carbon atom not involved with ring fusion. The numbering proceeds clockwise around the structure with hydrogenated carbon atoms.



Acenaphtylene **Fluoranthene** **Pyrene** **Perylene**

Fig. 8: IUPAC Parent compounds (examples) [**62**]

The outer sides of the rings are given letters in alphabetical order, beginning with the side between C_1 and C_2. The numbering of anthracene and phenanthrene are "retained exceptions" to this rule. To elucidate the nomenclature of PAHs, the name for benzo[*a*]pyrene is taken as an example (**Figure 9**). Benzo[*a*]- indicates that an aromatic ring is fused to pyrene in the *a* position. The ring can be fused also in positions *b*, *e*, and so on. However, positions *a*, *b*, *h* and *i* are equivalent, and so are *e* and *l*. Accordingly, there are only two isomers, benzo[***a***]pyrene and benzo[***e***]pyrene. Only the first letter is used, and the formulas are written according to the above rules. Also in positions *cd*, *fg*, and so on, of pyrene the ring can be fused.

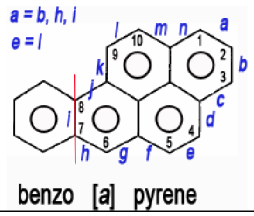


Fig. 9: Benzo[*a*]pyrene

The conjugated π-electron systems of the PAHs account for their chemical stability. They are solids at room temperature and have very low volatility. Depending on their aromatic character, PAHs absorb UV/VIS light and give characteristic fluorescence spectra (some compounds even exhibit phosphorescence). PAHs are soluble in many organic solvents, but they are very sparingly soluble in water, decreasing with increasing molecular weight.

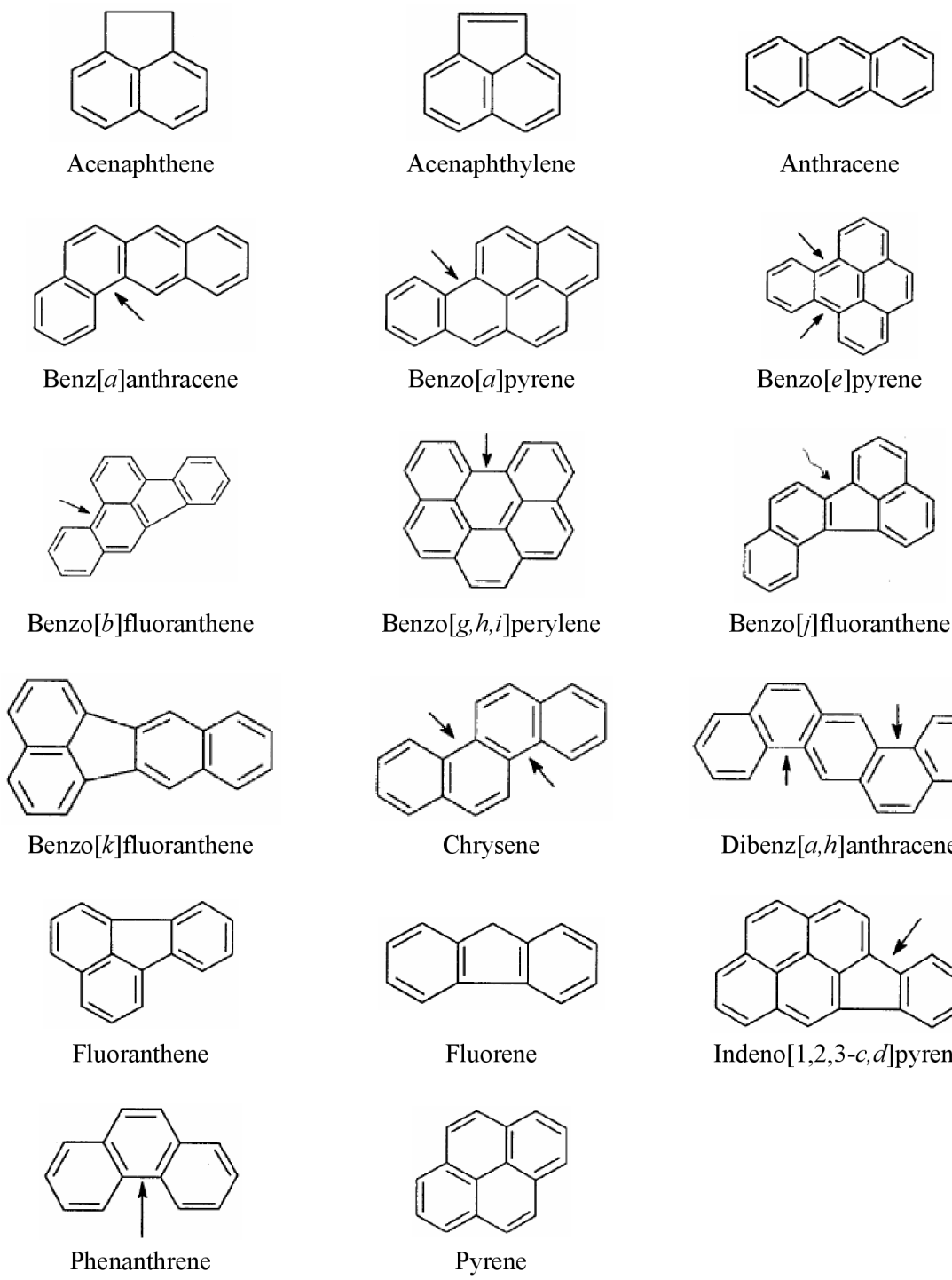


Fig. 10: Molecular structures of 17 selected EPA PAHs (⟶ bay region – see Toxicology, p. 22)

Chemically, PAHs react by substitution of hydrogen or by addition reactions where the saturation occurs. Generally, the ring system is retained. Most PAHs are photo-oxidised, a reaction which is important for the removal of PAHs from the atmosphere. The most common photo-oxidation reaction is formation of *endo*-peroxides, which can be converted to quinones. It has been found that the photo-oxidation of adsorbed PAHs can be greater than that of PAHs in solution. A significant chemical quality of PAHs is the ability to form derivatives (nitro- or chloro-derivatives). Some derivatives have exhibited stronger carcinogenic effects than PAHs themselves.

Tab. 7: Physical and chemical properties of selected PAHs [**64**]

Common name	CAS index	M [g/mol]	b. p. [°C]	water solubility [mg/L] (25 °C)	log K_{OW}	H*) (25 °C)
Acenaphthene	83-29-9	154.21	279	3.88[a]	3.92	18.5*
Acenaphthylene	208-96-8	152.19	280	3.93[a]	4.07[a]	12.7[a]
Anthracene	120-12-7	178.23	339.9	0.062[+]	4.45[+]	5.64*
Benz[*a*]anthracene	56-55-3	228.29	438	0.011[+]	5.79[+]	1.22*
Benzo[*a*]pyrene	50-32-8	252.31	495	0.0018[a]	5.97[+]	0.034
Benzo[*e*]pyrene	192-97-2	252.31	311	0.0073[a]	6.44[+]	0.02
Benzo[*b*]fluoranthene	205-99-2	252.31	481	0.0015	5.78	0.05[a]
Benzo[*g,h,i*]perylene	191-24-2	276.33	525	0.00014	6.90[+]	0.027[**]
Benzo[*j*]fluoranthene	205-82-3	252.31	480	0.0025	6.40	-
Benzo[*k*]fluoranthene	207-08-9	252.31	480	0.0011[a]	6.05[a]	0.042[**]
Chrysene	218-01-9	228.29	448	0.0019[+]	5.73[+]	0.53*
Dibenz[*a,h*]anthracene	53-70-3	278.35	524	0.00056[*]	6.50[+]	0.0075[*]
Fluoranthene	206-44-0	202.25	384	0.240[+]	5.16[+]	1.96*
Fluorene	86-73-7	166.22	295	1.90[+]	4.18[+]	9.81*
Indeno[1,2,3-*c,d*]pyrene	193-39-5	276.33	-	0.0023[a]	6.72	0.029[**]
Phenanthrene	85-01-8	178.23	340	1.10[+]	4.46[+]	4.29*
Pyrene	129-00-0	202.30	404	0.132[+]	4.88[+]	1.71*

[*]) [Pa m^3/mol], [a] approx., *mean value, [+]recommended, [**]20 °C

PAHs can be found almost everywhere, in the air, soils and water originating from natural and anthropogenic sources (see **Table 8**). The contribution from natural sources such as forest fires and volcanoes is minute compared to the emissions caused by humans. The burning of fossil fuels causes the main emissions of PAHs. Other contributions come from the combustion of waste and wood, and from the spillage of raw and refined petroleum. PAHs also occur in tobacco smoke and grilled, smoked and fried food. The most important source of PAHs in the air of the occupational environment is coal tar, formed by pyrolysis of coal in gas and coke works. A marked increase of traffic volume and a rapidly growing number of automobiles during last decades present a serious problem as for PAH emission, getting worse especially during the summer and winter smog situations.

Tab. 8: Important PAH sources [**63**]

Natural sources (minute)
✓ forest fires and volcanoes, crude and shale oil
✓ biosynthesis
Human activities
✓ burning of wood in homes (the largest single source)
✓ traffic emissions
✓ tobacco smoke
✓ gas cooking and heating appliances
✓ industrial processes, power generation
✓ municipal waste incineration, open waste burning
✓ food production

The commercial production of PAHs is not a significant source of these compounds in the environment. Some PAHs are used as an intermediate in dye production (anthracene); in the manufacture of pharmaceuticals and plastics (acenaphthene); or in the formation of polyradicals for resins, and in the manufacture of dyestuffs (fluorene).

Toxicology of PAHs

Data from animal studies indicate that several PAHs may induce a number of adverse effects, such as immunotoxicity, genotoxicity, carcinogenicity, reproductive toxicity (affecting both male and female offspring), and may also possibly influence the development of atherosclerosis. However, the critical endpoint for the health risk evaluation is the well-documented carcinogenicity of several PAHs [**67**]. The IARC classification of PAH carcinogenicity for humans is listed in **Table 9**. However, the carcinogenicity of individual PAH isomers may vary a lot. Early studies demonstrated that the structure of a particular PAH can be an important determinant of its biological activity [**71**]. For a number of PAHs containing so-called "*bay region*" including benzo[*a*]pyrene and dibenz[*a,h*]anthracene [**72**] (see p. 20), their carcinogenic potency strongly depends on regioselective metabolic activation toward bay region *anti*-diol-epoxides and further oxidized metabolites [**73**]. In contrast, the situation is less clear for PAHs that contain the structural element of a "*fjord region*", either alone or together with a bay region (dibenzo[*a,l*]pyrene, **Figure 11**).

Tab. 9: IARC classification of EPA PAHs [**45**]

Group	Compounds
1	Benzo[*a*]pyrene,
2A	Dibenz[*a,h*]anthracene, Dibenzo[*a,l*]pyrene,
2B	Benz[*a*]anthracene, Benzo[*b*]fluoranthene, Benzo[j]fluoranthene, Benzo[k]fluoranthene, Chrysene, Dibenz[*a,i*]pyrene, Indeno[*1,2,3-cd*]pyrene,
3	Acenaphthene, Anthracene, Benzo[*ghi*]perylene, Benzo[*e*]pyrene, Fluoranthene, Fluorene, Perylene, Phenanthrene, Pyrene

Human carcinogenicity groups:
(1) carcinogenic; **(2A)** probably; **(2B)** possibly; **(3)** not classifiable; **(4)** probably not carcinogenic

Unlike PCBs or TCDDs, PAHs are not included in the TEF scheme, despite an evidence for AhR binding. That is because of their short half-lives and relatively weak AhR activity. In addition, the role of the Ah receptor in their toxicity is uncertain. Furthermore, PAHs are DNA-reactive and mutagenic and these mechanisms play a large role in both the carcinogenicity and immunotoxicity of the PAHs [**65**]. In contrast, TCDDs and other dioxin-like compounds are not DNA-reactive. While there are PAHs that bind to the AhR, the role of the AhR or other competing pathways in the toxicity of these compounds has not been clearly defined. Attempts to derive relative potencies of individual PAHs (relative to benzo[*a*]pyrene) have been published, and the idea of summarising the contributions from each of the selected PAHs into a total benzo[*a*]pyrene equivalent dose (assuming additivity in their carcinogenic effects) has emerged [**66**]. However, individual PAHs have been shown to interact metabolically resulting in synergistic, additive or antagonistic effects.

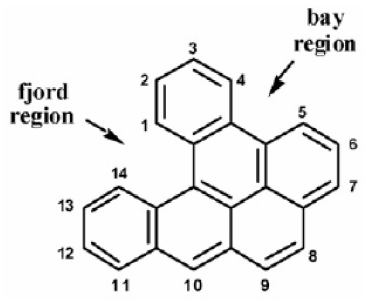


Fig. 11: Bay and fjord regions of dibenzo[*a,l*]pyrene

Metabolism of PAHs

The metabolism of PAHs has been studied extensively *in vitro* and *in vivo*. The enzyme systems that metabolize PAHs are widely distributed in the cells and tissues of humans and animals. The highest metabolising capacity is present in the liver, followed by lung, intestinal mucosa, skin and kidneys. Animal and human foetal tissues have the capacity to metabolise PAHs, but at a low rate compared to the adult tissues [**70**]. The microsomal mixed function oxidase system is an enzyme system primarily responsible for the PAH metabolism, which converts the non-polar PAHs into polar hydroxyl- and epoxy-derivatives [**68**]. Epoxides are the major intermediates in the oxidative metabolism of aromatic double bonds. The epoxides are reactive and enzymatically metabolised to other compounds such as dihydrodiols and phenols [**69**] (**Figure 12**). The mechanism of action of most PAHs involves covalent binding to DNA by PAH metabolites. The bay region diol epoxide intermediates of PAHs are currently considered to be the ultimate carcinogens for alternant PAHs. Once the reactive bay region epoxide is formed, it may covalently bind to DNA and other cellular macromolecules and presumably initiate mutagenesis and carcinogenesis (**Figure 14**). The *in vitro* formation of reactive, DNA-binding, free radicals of PAHs can be catalysed by a number of peroxidating systems, including microsomal prostaglandin synthetases, present in a variety of different tissues [**74**]. However, the significance of this pathway for PAH carcinogenicity in experimental animals and humans is as yet unclear [**75**].

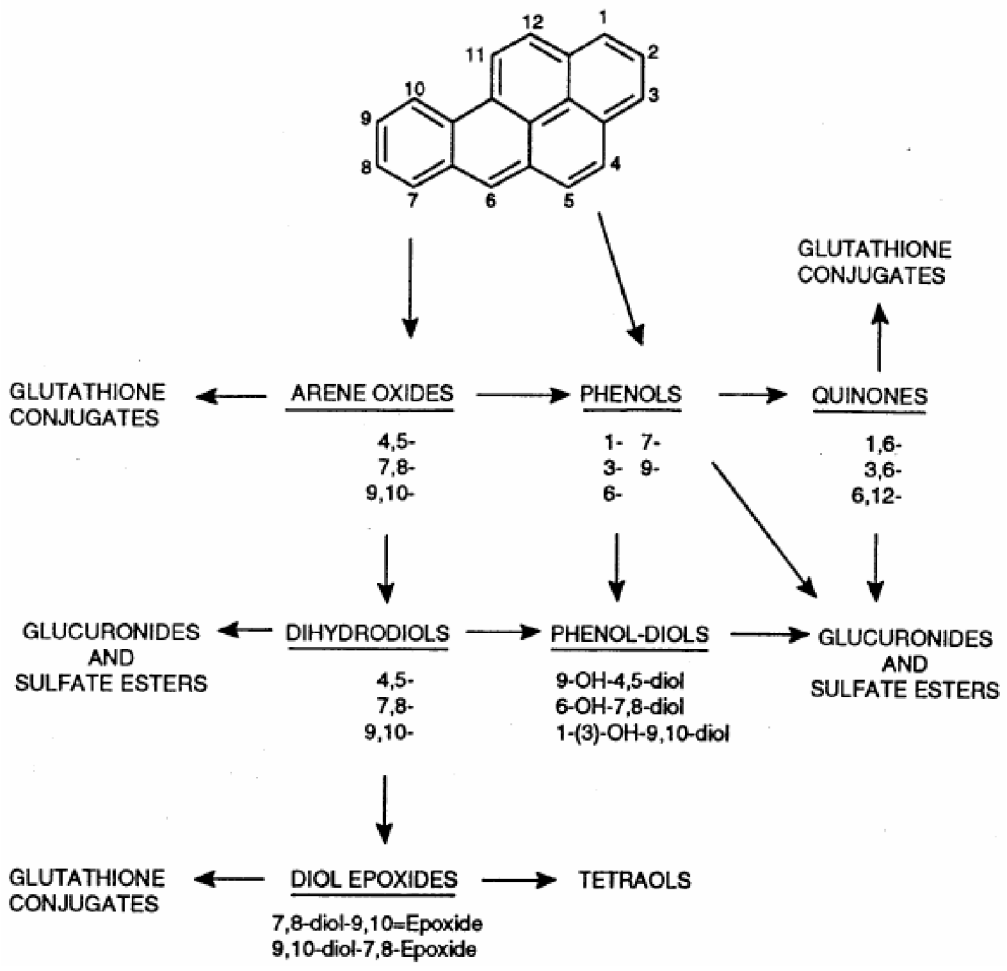


Fig. 12: Proposed metabolic scheme for benzo[*a*]pyrene [**63**]

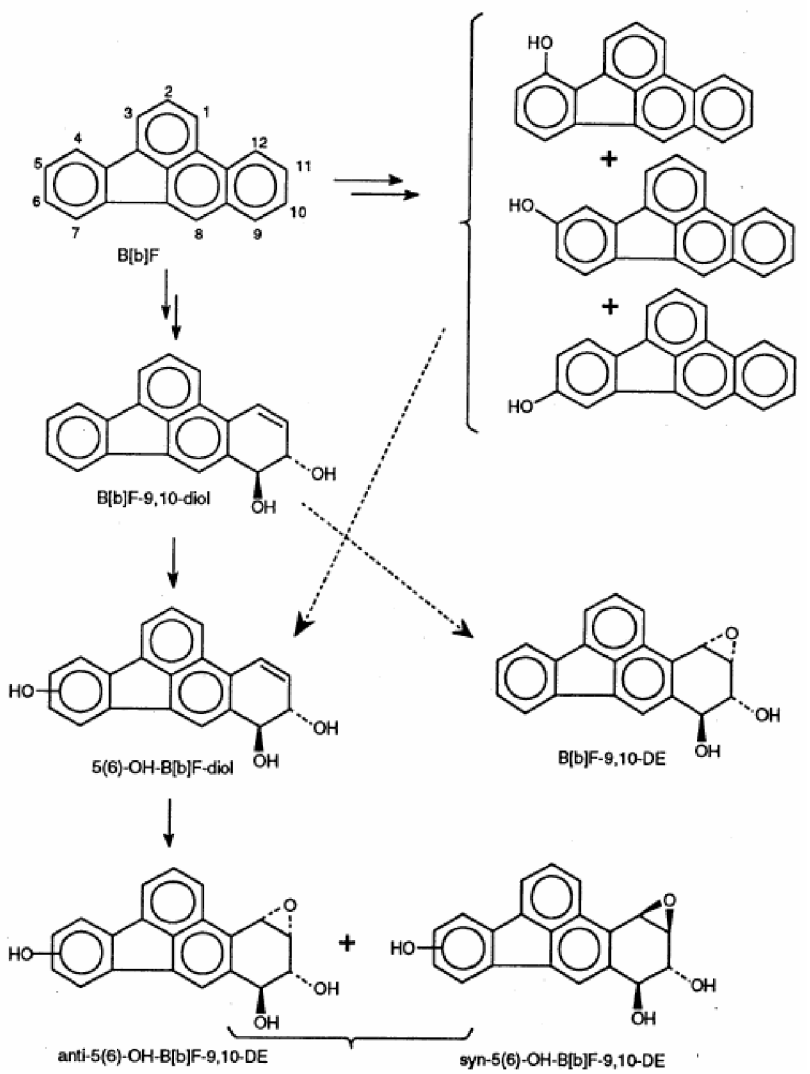


Fig. 13: Proposed metabolic scheme for benzo[*b*]fluoranthene [**63**]

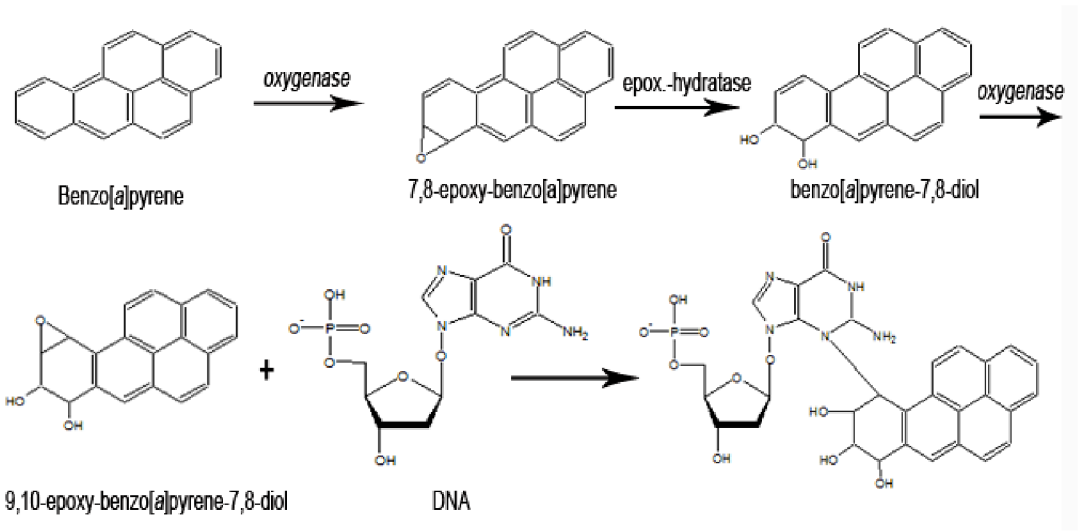
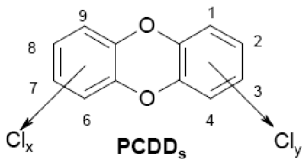


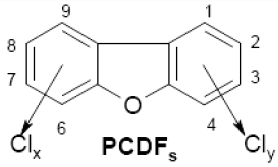
Fig. 14: Proposed PAH-DNA toxic effect of benzo[*a*]pyrene in mammals [**63**]

2.1.4. Other POP (PBT) compounds

Polychlorinated dibenzo-p-dioxins and dibenzofuranes (PCDDs/Fs)

represent an important and widely discussed group of environmental pollutants, posing a significant problem due to a very high toxicity of some of their representatives. PCDDs/Fs are high stable, resistant to breakdown and persist in the environment for long time. They are released into the air in emissions from municipal solid waste and industrial incinerators, vehicles, oil- or coal-fired power plants, burning of chlorinated compounds such as PCBs and some plastics (e.g. PVC), and cigarette smoke. PCDDs/Fs are not classifiable as to carcinogenicity to humans (cat. 3, IARC 1997), but the 2,3,7,8-TCDD congener **is** carcinogenic (cat. 1, IARC 1997) [**45**]. This congener is even one of the most toxic compounds (min. toxic dose for human is 0.1 $\mu g.kg^{-1}$). PCDDs (with PCBs and PCDFs) are included in the TEQ scheme (see p. 10) [**77**].





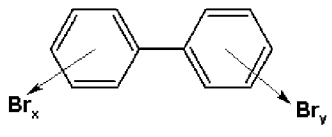
	Polychlorinated dibenzo-*p*-dioxins	**Polychlorinated dibenzofuranes**
(CAS)	(1746-01-6)**	(51207-31-9)++
P/U+	None/None	None/None
log K_{OW}	6.8**	6.53
sol. (25)*	$3.2x10^{-4}$ mg/L (estimated)	-

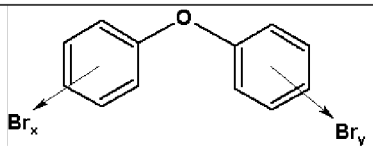
+ production/use today; * solubility in water at 25 °C; (x + y = 1-8)

The CAS numbers of **2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and ++2,3,7,8-Tetrachlorodibenzofuran

Polybrominated biphenyls and diphenyl ethers (PBBs/PBDEs)

are used as additive flame retardants (FRs) in a wide range of products including thermoplastics. These FRs are added to polymer materials instead of being chemically incorporated into the matrices and, as a result, they are much more prone to leaching or escape from the finished polymer product than reactive FRs. A widespread use has resulted in their ubiquitous presence in the environment. The toxicological properties of PBBs are very similar to those of structurally related PCBs (see p. 8). Some PBDEs may have some limited 'dioxin-like' activity. Furthermore, a formation of brominated dioxins and furans on combustion of PBDE containing products may be a hazard [**76**].



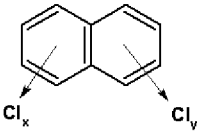


	Polybrominated biphenyls (PBBs)	**Polybrominated diphenyl ethers (PBDEs)**
(CAS)	(59080-40-9) (Hexa-PBB 153)	(68928-80-3 - HepBDE)
P/U+	None/None	Yes/Yes
log K_{OW}	9.10e	6.81+
sol. (25)*	0.011 mg/L	0.015+ mg/L

+ production/use today; * solubility in water at 25 °C; (x + y = 1-10); eestimated; +BDE 47

Polychlorinated naphtalenes (PCNs)

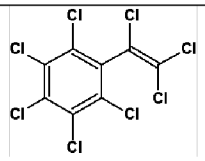
had been used in many applications till the end of the Second World War, but then PCNs began to be replaced by PCBs. Unlike PCBs, there is only little information on the PCN world production in the 20th century. An estimated output should approach 10% of the PCB production. PCNs continue to be detected in the environment, because of old loads and their persistence and bioaccumulation. They are structurally similar to PCDDs and some PCB congeners and their 'dioxin-like' toxicity mechanism is closely related to the Ah receptor. That is why PCNs are included in the TEF scheme.



(CAS)	(2234-13-1) **CN 75**
P/U$^+$	None/None
log K_{OW}	7.70

Octachlorostyrene (OCS)

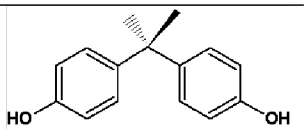
was never commercially produced. Rather, it was historically produced as an inadvertent by-product of high-temperature industrial processes involving chlorine such as the electrolytic production of chlorine gas or magnesium, the refining and degassing of aluminium smelt, and the chlorination process involved in e.g. tantalum production. OCS is persistent and bioaccumulative. Among its potential adverse effects, OCS has a potential to interfere with metabolism in fish and is highly toxic to aquatic organisms. However, the toxicological significance of the concentrations found in wildlife is not clear at this time, and its effects on humans are not well known as well.



	octachlorostyrene
(CAS)	(29082-74-4)
P/U$^+$	None/None
log K_{OW}	6.20
sol. (25)*	0.00174 mg/L (est.)

Bisphenol A (BPA)

is used as an intermediate in the production of epoxy resins and polycarbonate plastics. Additionally, BPA is a component of some specialty applications, such as flame retardanrs, and as an antioxidant and stabiliser in the production of PVC and other plastics. BPA is regarded as an endocrine disrupting chemical, which among others competitively inhibits estrogen from binding to its receptor. Studies have also indicated that BPA can act as an anti-androgen, blocking the androgen receptor-mediated effects of dihydrotestosterone in biological systems [**78**].



(CAS)	**BPA** (80-05-7)
P/U$^+$	Yes/Yes

2.2. Environmental Fate of POPs

The environment is continuously loaded with foreign organic chemicals (xenobiotics) released by urban communities and industries. However, the presence of a xenobiotic compound in a segment of the ecosystem does not, by itself, indicate injurious effects. Connections must be established between external levels of exposure, internal levels of tissue contamination and early adverse effects. When released into the environment, substances will be subject to transport and transformation processes. These processes, together with emission patterns, environmental parameters and physicochemical properties of the substances, will govern their distribution and concentration in environmental compartments such as water, air, soil, sediment and biota [**81**].

The atmosphere is the most important pathway, on which natural and anthropogenic organic chemicals are transported in the ecosystem and the wet and dry deposition events are important processes that remove these chemicals from it [**82**]. POPs are known to undergo long-range atmospheric transport (LRAT), travelling according to their physical-chemical properties and to the characteristics of the environment that they encounter (soil properties, climate, wind direction and speed and so forth), reaching remote regions where they have never been produced or used. This process can happen as a simple emission-transport-deposition event or following a series of "hops" by deposition onto a surface and subsequent emission (this process is also called *global distillation*) [**83**]. The transport distance and the number of air-surface exchange episodes depend on the type of surface (soil, water, vegetation, etc.) and on the physical–chemical properties of the given chemical. According to this principle, persistent chemicals with higher volatility will undergo the LRAT followed by deposition in distant areas (e.g. arctic regions), while those with lower vapour pressure will be deposited preferably in areas closer to the emission source. Many other mechanisms and factors are able to influence the distribution of POPs in the atmosphere: the capacity of the environmental compartments to accumulate or to degrade POPs; the atmospheric circulation patterns; the spatial and temporal distribution of primary sources and the kinetic of the air-surface exchange. In addition to that, present climate change may have the potential of affecting the behaviour and distribution of POPs. Direct effects of climate change can be very effective in altering the partitioning of POPs among the environmental compartments. POPs in the air can be degraded by photodegradation and via reactions with hydroxyl radical (OH·), both depending, to a certain extent, on the solar radiation intensity [**84**]. Moreover, studies dealing with this issue document a seasonal variability in the levels of POPs. During summer months there are up to five times higher atmospheric levels of these pollutants, which may relate to their volatility [**85**].

The ultimate sink for many of these contaminants is the aquatic environment, either due to direct discharges or to hydrologic and atmospheric processes [**79**]. In particular, the oceans play an important role in controlling the environmental transport, fate and sinks of POPs at regional and global scales. Even though POP concentrations in the open ocean are lower than those observed in coastal areas, the large oceanic volumes imply that they may represent an important inventory of POPs [**86**]. As for the continental ecosystem, a major amount of POPs (PCBs, DDTs etc.) is still deposited in freshwater sediments which are at the very beginning of the aquatic food chain and so they cause a consequent contamination of the following members of the food web (fish etc.), including humans. For example, an estimated PCB deposition in particular parts of the ecosystem is summarised in **Table 10** [**87**].

Tab. 10: An estimated total PCB deposition in the global ecosystem

Environment	PCBs (in tons)	PCBs in ecosystem (%)	Global production (%)
Continent and shore			
Air	500	0.13	
Rivers and lakes	3,500	0.94	
Sea water	2,400	0.64	
Soils	2,400	0.64	
Sediments	130,000	35	
Biota	4,300	1.1	
In total (A)	143,000	39	
Open ocean			
Air	790	0.21	
Sea water	230,000	61	
Sediments	110	0.03	
Biota	270	0.07	
In total (B)	231,000	61	
Total load of the environment (A+B)	374,000	100	31
Degradation/Burned	43,000		4
Disposal sites/in use	783,000		65
Global production	1,200,000		100

2.2.1. POPs in aquatic ecosystems

POPs have a tendency to partition between the air, water and organic phases, which permits a relatively easy movement from phase to phase, e.g. the cycling between the Earth's atmosphere and surface by deposition and evaporation. Atmospheric transport and the subsequent deposition are regarded as the main input of organochlorine compounds into the aquatic environment. The partition behaviour of these hydrophobic chemicals in sediment, water and biota is mainly determined by the lipid and organic carbon contents; the more hydrophobic the compound, the greater the partitioning to these phases [**91**]. The sorption of chemical matters to suspended particles which form sediments is a major process of removing POPs from the hydrosphere. Moreover, ocean and river currents play an important role in the sedimentation process; upper sediment layers in shallow streams with rather strong currents serve as such a 'temporary stop', unlike deeper and smoother streams where the final deposition of these compounds takes place. The water-sediment equilibrium, set over given period, can be expressed by $C_S = K_P \; x \; C_W$, where C_S refers to the concentration of given compound in sediments, C_W to the concentration in water, and K_P to the equilibrium constant reflecting the partition rate between the water-sediment phases. The content of organic carbon in sediments is also important due to its influence on the sorption of organic matters.

As for e.g. PCBs, it is generally true that the percentage of higher chlorinated congeners with higher K_{OW} coefficients in the total sum of PCBs increases through aquatic ecosystem levels as follows: sediment < plankton < fish < predators. However, it is also necessary to take some steric effects relating to especially octaCB congeners, which may result in lower PCB levels in fish as well as in plankton. In general, the concentrations of POPs in seas (and generally in water environment) are very low, normally at levels of pg (ng)/L [**93**]. However, the concentrations found in organisms forming aquatic biota and being at upper levels of the food chain are much greater. A significant increase in concentrations of some lipophilic xenobiotics in wildlife (esp. in fish), in comparison with the abiotic parts (sediments, water)

of polluted environment, have been observed since the 1960s. Subsequent studies dealing with the accumulation of both inorganic and organic matter have been primarily focused on (1) the protection of fish and other aquatic organisms against the exposure to these xenobiotics, and (2) the protection of humans and consumers, respectively [**92**].

Bioaccumulation

Persistent hydrophobic chemicals (PCBs, OCPs) may accumulate in aquatic organisms through two different mechanisms. The first one presents the direct uptake from water by gills or skin, by simple passive diffusion across a cell membrane (i.e. non-dietary uptake, **bioconcentration**). The whole process of bioconcentration results from the physical-chemical equilibrium between an organism and surrounding water, and particularly depends on the morphologic and physiological properties of gills. The second mechanism is called **biomagnification** and the accumulation of contaminants takes place via uptake of suspended particles (ingestion), and via the consumption of contaminated food and the following digestion. Biomagnification is related to the food habits and to the position of individual species within the food web. Furthermore, this process is especially conditioned by specific mechanisms of food intake in the digestive tract. Total intake and the accumulation of particular xenobiotics in an organism are given by both the mechanism and it is generally true that:

Bioaccumulation = Bioconcentration + Biomagnification

Contaminant levels in biota are determined primarily by the uptake and elimination kinetics, which are typical for both chemicals and organisms. A model of the processes governing bioaccumulation (uptake and clearance) in aquatic organisms is presented in **Fig. 15**.

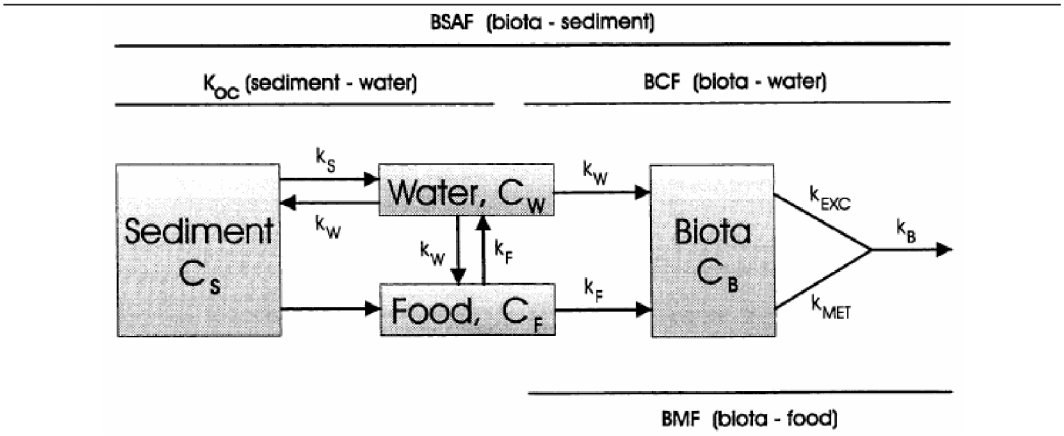


Fig. 15: Bioaccumulation model for aquatic organisms. The digestible sediment fraction is considered to be part of the food [**80**]. *See the following text for details.*

According to this model, the concentration of a chemical in biota (C_B) over time (t) can be expressed by:

$$\frac{dC_B}{dt} = \left[k_W C_W + k_F C_F\right] - k_B C_B = \left[k_W C_W + k_F C_F\right] - \left[k_{EXC} + k_{MET}\right] C_B \qquad (1)$$

Here, C refers to a concentration; k to a rate constant; and the subscripts W, F, B, EXC and MET to water, food, biota, excretion and metabolism, respectively. Uptake of organic pollutants in fish may be direct via exchange with the water phase ($k_W \cdot C_W$) or indirect via the consumption of contaminated food ($k_F \cdot C_F$). Although biotransformation of organic trace pollutants ($k_{MET} \cdot C_B$) has been reported for fish, clearance mainly occurs by simple release from the (lipoid) gill membranes and via faecal excretion into the surrounding water ($k_{EXC} \cdot C_B$) [**94**]. Since the intake via contaminated food is regarded as a minor way and thus can be ignored, the bioconcentration represents the majority process of xenobiotic intake in fish (i.e. directly from water) [**95**].

Bioconcentration

The term of *bioconcentration* refers to the accumulation of chemical compound caused by direct transfer from water and depends on the concentration of this compound in water, on the K_{OW} factor, and on the lipid content in animals [**96**]. Two major approaches towards the quantification of both the bioconcentration and the bioaccumulation, using the *bioaccumulation (BAF)* and the *bioconcentration (BCF)* factors, are employed at present. These factors express the ability of particular compound to penetrate and to be cumulated in given organisms. Both the factors can be defined and measured under steady state conditions (after an equilibrium between intake and excretion is reached) – the *static concept* (BAF_S and BCF_S) – or are expressed as a time function, depending on the actual degree of intake and elimination of compounds, and on the metabolic activity – the *dynamic concept (BAF(t)* and *BCF(t))*.

The *bioconcentration factor* (BCF) of a chemical is the ratio of its concentrations in the organism and in water during steady state or equilibrium [**88**]. For the partitioning of chemicals between water and the lipid phases of organisms, the steady state BCF is defined as:

$$BCF = \frac{k_W}{k_B} = \frac{C_B}{C_W} \qquad (2)$$

Uptake of chemicals in organisms from water probably follows a passive diffusion mechanism analogous to that of oxygen uptake. Decreased oxygen levels in water result in a more rapid uptake and an increased body burden of hydrophobic chemicals [**97**]. A significant correlation between the uptake constant (k_W) of organochlorine compounds and fish oxygen consumption, regardless of fish size and species, was demonstrated [**98**]. The uptake rate depends on the water concentrations, which will generally be higher for less hydrophobic compounds. The rate of uptake of hydrophobic chemicals in fish increase with a higher lipid content of the biological membranes.

The fate of chemicals is largely determined by sorption to suspended particulates and sediments. Sorption depends on the characteristics of both the sediments and the chemicals involved [**90**]. If sorption of hydrophobic chemicals is considered as a partitioning between water and the organic fraction of sediment, then the *equilibrium sorption coefficient* (K_{OC}) can be expressed as:

$$K_{OC} = \frac{k_W}{k_S} = \frac{C_S}{C_W} \qquad (3)$$

Here the subscripts S and W refer to sediment and water, respectively. If the processes of bioconcentration and sorption on sediments have both reached equilibrium, then the ratio between BCF and K_{OC} can be combined to define the *biota-sediment accumulation factor* (BSAF):

$$BSAF = \frac{C_B}{C_S} = \frac{BCF}{K_{OC}} \qquad (4)$$

Both the BCF for the partitioning of chemicals between water and the lipid phases of organisms and the K_{OC} for the partitioning between water and the organic fraction of the sediment depend upon the hydrophobicity of the chemicals. The BSAF is used as a simple model to predict the bioaccumulation of neutral organic compounds, sorbed in sediments, in invertebrates and fish. However, there is an evident disadvantage of this approach, because of a significant relation between the xenobiotic availability and the chemical composition of sediments. Hence such a "normalisation" of the compound content taking the organic carbon content in sediment into consideration is used. Then the BSAF is modified as:

$$BASF = \frac{(C_t / L)}{(C_S / TOC_S)} \qquad (5)$$

where C_t refers to the tissue concentration of a compound (in μg/kg), L to the tissue lipid content (in g of lipids per gram of tissue), C_S to the sediment concentration of a compound (in μg/kg), and TOC_S to the total organic carbon content in the sediment (g of carbon/g of sediment).

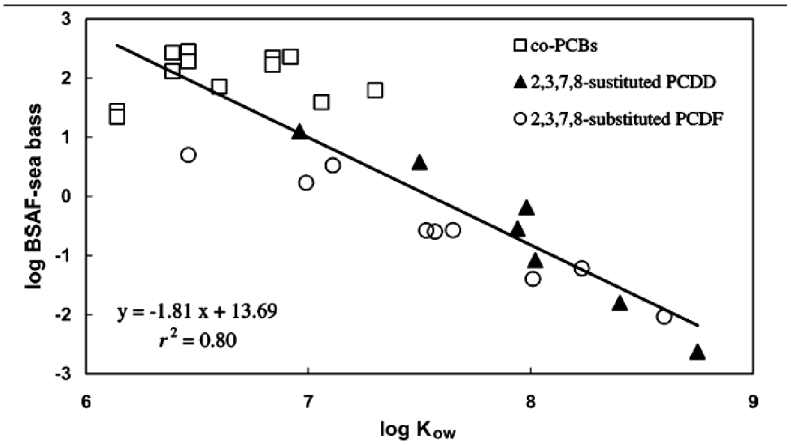


Fig. 16: The relationship between ocatanol/water partition coefficient (*log* K_{OW}) and the *log BSAF* values for 2,3,7,8-substituted PCDDs/DFs and co-PCBs. K_{OW} values were derived based on [**100**] for PCDDs/Fs and [**101**] for *co*-PCBs.

Higher chlorinated (Cl ≥ 7) and highly lipophilic PCB congeners (*log* K_{OW} ≥ 7) having structural barriers in bioaccumulation also have lower BASF values which follows a negative *BASF-log* K_{OW} correlation characterising the compound lipophilicity. The *BASF* value depends on the type of organic carbon (both chemical and biological) in sediment and on pollutant concentration. Despite high concentrations of higher-chlorinated congeners, the bioavailability for fish and shells is very low [**102**].

Biomagnification

Biomagnification is a ratio between the uptake of the chemicals from food and their clearance. During steady state, the biomagnification factor (BMF) can be defined as:

$$BMF = \frac{k_F}{k_B} = F_F \frac{E_F}{k_B} \qquad (8)$$

Here, F_F refers to the amount of food transported through the intestines per gram of fish per day and E_F to the efficiency of uptake of the chemical from food.

Since bioaccumulation of persistent and extremely hydrophobic compounds cannot always be explained satisfactorily by simple partitioning processes between sediment, water and fish, it is likely that the uptake via contaminated food (biomagnification) contributes significantly to the bioaccumulation of these contaminants in fish. If a primary source of chemical input to an aquatic ecosystem is a slow release from polluted sediments, then it is plausible that uptake by benthic organisms followed by predation by larger organisms such as fish may be a significant source of bioaccumulation [**89**]. Biomagnification only becomes a significant contribution to the overall bioaccumulation for compounds with the *log* K_{OW} values higher than 5.5. As for the other compounds (*log* K_{OW} < 5.5), the intake via contaminated food is regarded as minor.

For simulating the partition of lipophilic compounds between water and animal tissue a model bled n-octanol/water has been chosen, which is characterised by the K_{OW} partition coefficient. The BCF-K_{OW} relationship roughly predicting the extent of lipophilic compound bioaccumulation can be defined as:

$$\log BCF = a\, x \log K_{OW} + b \qquad (9)$$

It has been suggested that if the POP concentrations in the lipid of aquatic organisms are at equilibrium with water concentrations, simple one-to-one log-linear relationships between BCF (using lipid-corrected data for the organism) and K_{OW} should be observed. However, BCFs of very hydrophobic POPs (*log* K_{OW} > 6.0) have been shown to deviate below this 1:1 relationship in laboratory experiments and field measurements [**104**]. An analysis of a large data set of BCF values found that the BCF-K_{OW} relationship was linear for POPs with a *log* K_{OW} range of 3-6, but that a curvilinear model provides a stronger fit when very hydrophobic chemicals were included [**105**]. The *BCF-*K_{OW} relationships observed for zooplankton *C. hyperboreus* are presented in **Fig. 17.** Log BCFs of POPs that are substantially higher than those predicted by *log* K_{OW} have been shown to be due to trophic transfer, i.e., dietary accumulation. Hence, BCF is not a good predictor of

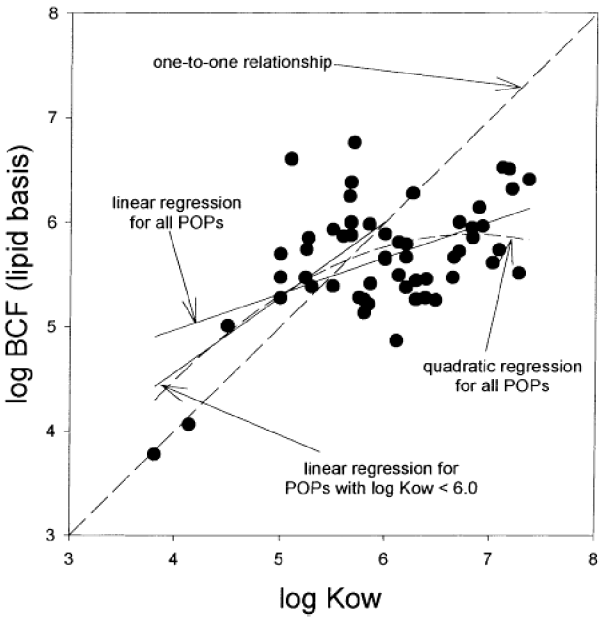


Fig. 17: The log BCF-log K_{OW} relationships for zooplankton *C. hyperboreus* [**103**].

hydrophobic POP concentrations in aquatic organisms for which dietary accumulation results in biomagnification.

Bioaccumulation is a very complex process, in which various mechanisms of intake, excretion, passive secretion and metabolism of xenobiotics are involved, and which can be influenced by the following physical-chemical and biochemical factors [**99**]:

- **Lipophilicity** – not only increases the overall bioaccumulation, but it also allows even more effective intake and penetration through those biomembranes of lipophilic nature. On the other hand, when the water concentration of a given compound declines, its effective elimination is negatively impacted
- **Molecule shapes and sizes** – M_r > 500-1000 and/or molecule diameters above 1 nm strongly limit the membrane penetration as well as the steric barrier.
- **Biochemical degradability** – both metabolic activity and excretion decrease the extent of bioaccumulation (the activity of detoxification systems in fish is rather weak)
- **Metabolite characters** – they are more hydrophilic in most cases, which allows excretion and decrease bioaccumulation
- **Bioavailability** – xenobiotics can be adsorbed on suspended particles (organic carbon, resp.) and such complexes are not easy to penetrate via biomembranes
- **Lipid content in microorganism** – higher lipid content intensifies bioaccumulation
- **Organism nature** – interspecies and intraspecies differences, age and sex differences, food chain position
- **Environmental factors** – e.g. temperature – rising temperature increase the extents of intake, metabolism and elimination, which may be caused by changes in biomembrane properties and in enzymatic activity

Bioavailability

When measuring bioaccumulation behaviour, the bioavailability of a substance considered is the crucial parameter for valid results. The bioavailability is defined as the fraction of the bulk amount of a chemical present in soil/sediment and (interstitial) water that can potentially be taken up during an organism's lifetime into an organism's tissues (excluding the digestive tract). When the concentration in fish is not related to the real bioavailable concentration in the water, this might result in underestimation of the bioconcentration potential. Deviations in the BSAF values predicted with the partitioning models may thus partly be due to differences in bioavailability of the chemicals, possibly resulting in a pronounced site-specific variation in bioaccumulation profiles of certain contaminants [**80**]. The bioaccumulation of e.g. PCBs, pesticides and PAHs can be affected by the presence of particles in the aquatic phase, such as sediment, humic acids and other dissolved organic matter (DOM). It was suggested that a reduction of the uptake of hydrophobic chemicals was caused by a reduced bioavailability of the compounds due to sorption on particles. On the one

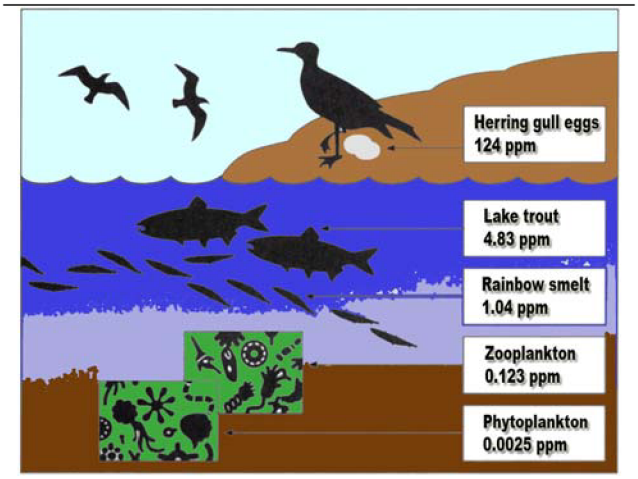


Fig. 18: PCB bioaccumulation and biomagnification in aquatic food chain. Great Lakes, Canada [**106**].

hand, distinct decreases in bioaccumulation of very hydrophobic contaminants due to DOM with a high binding capacity occurred at environmentally representative DOM concentrations. A pollutant uptake via sediments will only contribute significantly to the body burden of organisms which are able to digest (parts of) the sediment. This uptake route, which is probably of minor importance in fish, is represented in the bioaccumulation model of **Fig. 15** as a dotted arrow between sediment and food [**107**, **108**].

Eliminating processes (e.g. active excretion, biotransformation, losses by passive diffusion through membranes) naturally influence the bioaccumulation of xenobiotics in organisms which leads to decrease in bioaccumulation extent.

2.2.2. Biomarkers (bioindicators) of the environmental pollution

The contamination of water ecosystems can be determined by direct measurement of pollutant concentrations in water, sediment or biota. Yet, water levels of micro-pollutants with high *log* K_{OW} 5-7 (PCBs, OCPs etc.) are extremely low and hence hardly measurable. Even direct determination of these compounds in sediments often meets problems. The use of aquatic organisms (i.e. biota), mainly fish and invertebrates (bivalves, shells), is an alternative and useful approach. The bioaccumulation of certain persistent environmental contaminants in animal tissues may be considered to be a biomarker of exposure to these chemicals [**109**]. However, there is an array of authors/researchers often confusing relevant terms. Body burdens are not considered to be **biomarkers** or **bioindicators** since they do not provide information on deviations related to 'health'. In order to avoid confusion, the analytical/chemical indicators (body burdens) should be referred to as **bioaccumulation markers**, while all biological (biochemical, physiological, histological and morphological) indicators measured inside an organism or its products to as **biomarkers** [**110**]. Exposure assessment has to provide information on steady-state concentrations of potentially toxic xenobiotics in a selected environmental compartment. Exposure assessment by measuring contaminant levels in biota or determining the critical dose at a critical site (bioaccumulation) is called **bioaccumulation monitoring (BAM)**, including measurement of levels of chemical agents and their metabolites and/or derivatives in cells, tissues, body fluids or excreta.

Aquatic bioaccumulation markers

Fish are considered to be an ideal species for environmental pollution assessment, because of very low activity of detoxification enzyme systems. Notably as to hydrophobic xenobiotics, fish allow a rather correct reflection of source contamination profiles without any degradation changes and, moreover, they accumulate POPs in a broad extent unlike other alternative monitoring mediums (sediment, water). In order to assess aquatic ecosystem burden, fish are used in two different approaches. The first method, *active biomonitoring (ABM*, see BAM above), employs fish kept in a non-contaminated area and then transferred to the monitored area for a given period of time, where a gradual accumulation takes place. Analyses of contaminant levels in fish tissues are then in determinate intervals. This first approach primarily offers information on the current state of the environmental contamination. The second method, *passive biomonitoring (PBM)*, uses fish or water biota occurring naturally in specific polluted area. Both methods often complement each other. For the passive monitoring purpose, species specific for a particular locality and with a low river migration are used.

Biomonitoring should reflect changes in levels of water pollutants. For the interpretation of ABM or PBM method data, several important factors which can influence bioaccumulation in fish bodies have to be taken into account:

- **Species**
  - metabolic activity (possible degradation of xenobiotics)
  - lipid content (as tissue/lipid weight)
  - food web position and nutrition (biomagnification process)
- **Tissue/organ** ☹ different distribution of xenobiotic in muscle tissue, liver, skin and gonads (transport through organism, metabolic activity influence, lipid content etc.)
- **Gender** ☹ possible partial decontamination when spawning
- **Age** ☹ exposition length (possible contaminant concentration "dilution" per weight unit owing to growth)

Another technique, used worldwide for monitoring organic pollutants, is known as SPMD or semipermeable membrane devices. Since the first publication in 1990, nearly 200 studies have been reported, and SPMD is now the most mature technique for sampling organic pollutants. A simplified model of the main processes involved in the partitioning of chemicals among different environmental compartments (and SPMD) is presented in **Fig. 19** [**111**]. SPMDs are designed to mimic the bioconcentration process in aquatic organisms, and they can be also used for determining lipophilic compounds in sediments, soils or air. In general, lipid-containing SPMDs are placed in the water column or in sediments of lakes or rivers for periods of up to 60 days. Toxic contaminants present in the ecosystem permeate through the polyethylene membrane of the device and are sequestered in the lipid. Upon return to the laboratory, contaminant residues are recovered by solvent dialysis, while less than 5% of the lipid permeates through the walls [**112**]. SPMDs enables researchers to measure not only the presence, but also the bioavailability and bioconcentration potential of organic contaminants. Furthermore, in contrast to most living organisms, they can be exposed to harsh environmental conditions for long time periods and still remain operative [**113**]. Among the several designs of membrane samplers that have been used, triolein-containing SPMDs proved to be most effective in their capacity to accumulate lipophilic substances. The molecular size-exclusion limit of the polyethylene membrane is similar to that of biological membranes while triolein constitutes a significant fraction of the lipid pool of most aquatic organisms [**114, 115**]. SPMDs are often referred to as such a "*virtual fish*".

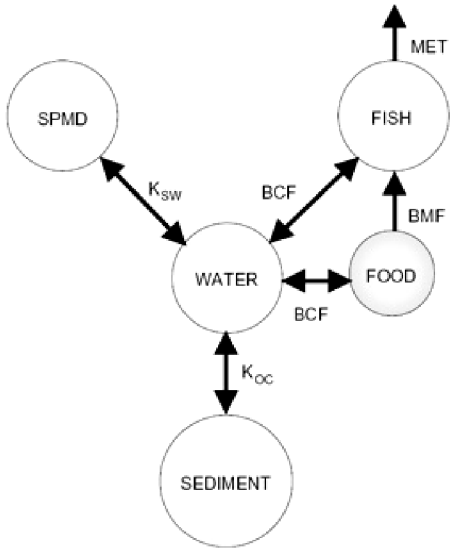


Fig. 19: Simpl. model of the processes governing the partitioning of chemicals in the aquatic environment; SPMD–water partitioning coefficient

Terrestrial bioaccumulation markers – birds of prey

Birds have extensively been used in the past as bioaccumulation markers of environmental contamination with persistent organic pollutants. They are situated high on the food chain, thus accumulating high levels of organohalogenated pollutants, and they are sensitive to environmental changes. The amount of organohalogenated pollutants accumulating within the tissues of birds is related to their diet and corresponding trophic position, but also to differences in accumulation among habitats and ecosystems (marine vs. terrestrial) [**117,118**].

However, it is difficult to use birds for an assessment of pollutant transfers within the food web, except for those species partly associated with the aquatic habitats where the transfer can be documented in some measure. These birds are known to live mainly on fish, hence tend to accumulate relatively high concentrations of PCBs and other POPs in the lipid tissue. For instance gulls, cormorants or herons are fish feeders, and thus are top predators in aquatic environments; therefore, they are a suitable bioindicator species [**116**]. They differ from true terrestrial species, such as gallinaceous birds, which generally have lower concentrations of POPs. However, for eagles, hawks, owls and other raptors, which are at the top of their respective food chains, there is a potential for high exposure due to their magnification potential (i.e., 10,000 and higher) and their unique feeding habits. Those individual species that possess feeding strategies in which they hunt other birds (or their eggs) and mammals – which already may be exposed and also have biomagnified these contaminants – will have the highest exposures and will possibly move past an effects threshold. Additionally, for birds that are scavenger feeders the potential for exposure is high, though dependent on the frequency of such behaviour [**119**].

Because practical and ethical reasons, low numbers, and often legal protection inhibit the sacrifice of free-living animals, methods for non-destructive biomonitoring have been developed. The use of hair, a keratinous tissue, has recently been evaluated as a method for the analysis of persistent organic pollutants [**120**]. In contrast to hair, which is continuously growing, feathers grow only for a certain period of time and are connected to the blood stream (and its circulating pollutants) only during this limited time period. While many biomonitoring studies on organic pollutants have previously focused on bird eggs, feathers have the advantage that they can be collected irrespective of season, age or sex. Moreover, since one feather can easily be removed from a living bird without causing severe damage, this technique could be valuable as a non-destructive biomonitoring tool for endangered species [**121**]. Another simple approach consists in the use of specimens found dead.

As stated previously, birds of prey are very suitable to study the bioaccumulation in specific target organs. E.g., PCB levels in unhatched eggs of Goshawks from Northern Germany have decreased since the 1970s and are relatively constant during the last 8–10 years [**123**]. This trend is also visible in gull eggs from the Great Lakes [**124**]. In contrast, the levels of PBDEs (which are becoming a similarly serious problem like PCBs recently) in herring gull eggs from the Great Lakes region have risen exponentially [**125**]. This general increase of PBDE levels is also detectable in fish, marine mammals and humans.

Recent findings (2006) of PCBs in bird species from Switzerland are presented in **Fig. 20**. These results are, according to the authors, comparable to congener patterns found e.g. in eggs from Norwegian predators, in tissues from British birds and in fulmars from the Faeroe Islands [**122**].

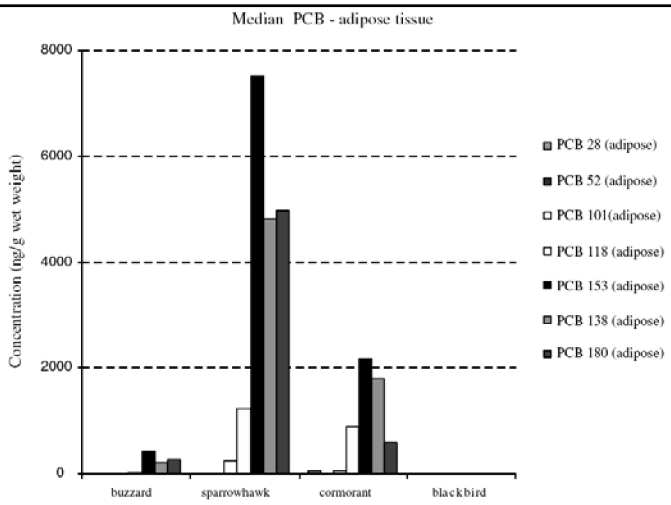


Fig. 20: Median PCB patterns in adipose tissue of birds of prey and blackbird from Switzerland [**122**].

As for cormorants, whose contamination assessment should be included in this study, a few studies have been published. Higher levels of OCPs and other chlorinated pollutants were detected in muscle tissues and in other organs of cormorants from the Danube Delta, Romania (1600-5000 ng/g lw; the present levels of DDTs in cormorant tissues are lower than those (mean 58,800 and 12,400 ng/g lw) measured in cormorant eggs samples in 1982 and 1997, respectively). This may suggest that the pollution level with DDTs has seriously decreased in the Danube Delta due to the effectiveness of DDT ban in 1995. However, for PCBs, while a decrease was observed between 1982 and 1997 (23,600 and 1500 ng/g lw, respectively), a steady state is observed for the present samples (range 690-3500 ng/g lw), suggesting that only a very slow PCB degradation occurs in the region. The PCB concentrations in cormorants from the Danube Delta are much lower than concentrations (between 13,000 and 63,000 ng/g lw) measured in liver of cormorants from the Southern Baltic Sea [**127**]. A Greek study (**Fig. 21**) brings the fingerprint of OCPs in cormorant eggs, where the relative proportions of β-BHC and *o,p'*-DDD were the highest. However, the authors conclude that levels of OCPs and PCBs found in the samples are too low to have any biological implications on the cormorants [**128**].

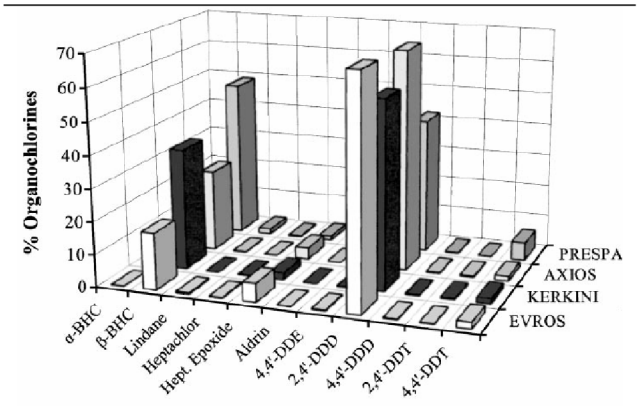


Fig. 21: Fingerprint of organochlorine pesticides detected in cormorant eggs in four wetlands areas in Greece [**128**].

In the Czech Republic, the contamination of unhatched eggs from raptors and owls was monitored in the Southern Moravia and seven indicator PCB congeners were detected. After considering the results, no intra- or interspecies differences were found. In particular, more chlorinated PCBs (esp. PCB 153) were detected the most, which corresponded to the findings from Germany, Canada or the USA [**126**].

2.3. POPs and Analytical Approach

POPs represent a large group of different chemical compounds and there is no doubt that their analysis is a complex and challenging issue. Dozens of PCB congeners, organochlorine pesticides and many other halogenated pollutants (PBDEs, HCHs etc.) occur in the environment samples on very different levels (and often in trace concentrations), which significantly complicates the whole analytical process. An accurate determination of critical xenobiotic concentrations, especially in biota and humans, is a crucial step towards a right toxicological assessment of exposure risks and dose/effect ratios. Low levels of target pollutants in the samples also make great demands on the analytical methods themselves, which must meet adequate requirements for precision, sensitivity and selectivity.

The analytical chemistry of POPs has been going through a rapid development since the 1960s, when adverse effects of these pollutants on the environment and the human health were discovered and when demands for ever more sophisticated analyses began to increase sharply. Some methods and approaches soon proved unsuitable or insufficient and they have been replaced or abandoned. For example, packed columns used in the gas chromatography determination of PCBs showed to be unable to achieve required resolution of all congeners, which made their right quantification impossible. Their determination was based on a simple comparison between the areas (or heights) of majority peaks in an industrial PCB mixture and in a real sample, which naturally led to incorrect results. And later, the introduction of silica capillary columns brought about such a small revolution not only in the POP analysis.

Fig. 1A illustrates the various generic steps of such an analytical procedure before the toxic pollutants undergo quantitative analyses. **Fig. 1B** shows a selection of different gas chromatographic techniques that have been applied in environmental toxicant analysis [**129**]. In the following chapters, each single step of this analytical procedure will be described and discussed in detail.

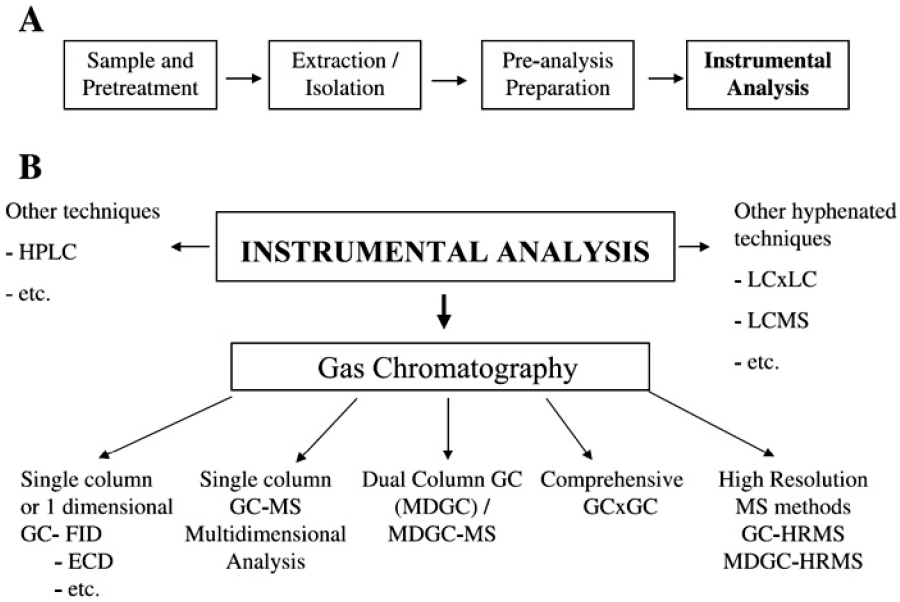


Fig. 22: (A) Generic steps in the analytical procedure. The figure, however, does not show the specific manipulations such as internal standard addition, etc. (B) Gas chromatographic techniques that are available for toxicant analysis [**129**].

2.3.1. Sampling procedures and techniques

Sampling is the first and limiting step in the whole analytical process, and has a significant influence on the accuracy of the final results. In this activity it is considered indispensable to ensure the representativeness and integrity of the sample during the entire sampling process. Additionally, quality requirements in terms of equipment, transportation, standardization, and traceability are indispensable. It is important that all sampling procedures are agreed upon and documented before starting a sampling campaign. The analyte, matrix, sampling site, time or frequency, and conditions should be determined depending on the objective of the sampling. There are lots of regulations and directives, how to treat samples, following the GLP (Good Laboratory Practise) and the QA/QC processes.

Water & sediments

It is generally accepted that fine-grained suspended and bottom-sediment particles (silt and clay) accumulate greater concentrations of contaminants. The assessment of sediment quality must therefore be carried out on the fine-grained sediments sampled in areas of the water body where permanent accumulation of sediments is taking place [**130**]. In particular, *corers* and *bottom samplers* are used for fine-grained sediments, *grab samplers* are used for collecting fine-to-medium grained bottom deposits from a depth of 15-20 cm. Water samples are mainly collected into cleaned *glass* or *plastic (PE) containers*. In general, *glass, porcelain, stainless steel, Teflon, or Teflon-coated* instruments should be used in handling sediment samples to be analyzed for organic components. Homogenized sediment samples are mainly stored at 4 ºC until analysis.

Besides these *active* sampling methods (direct sampling), there is an array of 'indirect' or *passive* techniques, which are generally based on free flow of analyte molecules from the sampled medium to a receiving phase in a sampling device, as a result of a difference between the chemical potentials of the analyte in the two media. The net flow of analyte molecules from one medium to the other continues until equilibrium is established in the system, or until the sampling period is stopped [**131**]. Sampling proceeds without the need for any energy sources other than this chemical potential. Passive samplers usually combine sampling, selective analyte isolation, pre-concentration and, in some cases, speciation preservation in one step. A review of passive samplers used for monitoring pollutants in various media has been published [**132**], including *SPMD* (see Chapter 2.2.2.), new and useful *SPME* (solid-phase microextraction), *LDPE and silicone strips* and many others.

Biota

The collecting of biota samples is naturally more difficult and requires a specific approach. Fish and other aquatic animals can be caught by various types of *nets* or by *electrical fishing*, based on using electrical devices for stunning animals in water, or as described in the Chapter 2.2.2., "non-contaminated" fish are kept in *cages* for a period of time in order to monitor the bioaccumulation process (*active biomonitoring)*. When monitoring pollutants in bird species, mainly animals found dead or unhatched eggs are used. *Shooting* or other ways of hunting are used rarely, esp. within the legal control of bird population. In the Czech Republic, all raptor species are protected, however, they are sometimes being shot/hunted illegally by gamekeepers or professional fishermen.

2.3.2. Matrix preparation, Extraction/Isolation

Prior to the isolation of target analytes from the sample, homogenization and/or grinding to rupture cell membranes appear to be the most commonly used pre-treatment procedures for tissue matrices (e.g., fish muscle tissue). Soil and sediment samples are usually dried and then undergo sieve analysis to obtain the specific particle fraction suitable for the following extraction. Bigger pieces (sticks, stones, etc.) are removed before sieving. Water samples could be filtrated prior to analyte extraction, but this step is not required.

The extraction of analytes from the sample matrix into the extraction matrix is generally regarded as one of critical steps in the whole analytical process. Environmental (or real) matrices contain many compounds so the sample preparation requires a selective extraction in order to obtain, ideally, target analytes only. The concentration of hydrophobic lipophilic pollutants (PCBs, OCPs, etc.) in environmental matrices is highly dependent upon the lipid content of the material. It should be noted that the concentration of the planar or non-*ortho*-substituted PCBs, which are considered the most toxic PCB congeners, is generally ~ 1000-fold lower (ng/kg) than those of non-planar or *ortho*-substituted PCBs (mg/kg). In addition, other compounds, such as pesticides, lipids, biological material, or chlorophyll from plants, are also extracted and can interfere with the analysis. Thus, additional fractionation steps are included in their clean-up [**137**, **138**].

In fact, extraction techniques used for both sediment (soil) and biotic samples do not vary a lot. Classical (or conventional) methods, which have been used for decades, include Soxhlet extraction, ultrasonic extraction (sonication) and liquid-liquid extraction (LLE, for liquid samples). However, their drawbacks (time consumption, large volumes of expensive solvents, difficult automation) made scientists develop new extraction techniques for POPs, which are more economical, effective and relatively clean. Let us mention ASE (Accelerated Solvent Extraction, also known as Pressurised - PSE), MAE (Microwave Assisted Ext.), SFE (Supercritical Fluid Ext.) or SP(M)E (Solid Phase (Micro)Ext.). Comparison of traditional and recent extraction techniques is in **Table 11**.

Shaking and Ultrasonic extraction (USE)

The simplest extraction technique for solid matrices is to blend or ultrasonicate the sample with an appropriate organic solvent at room temperature. Apart from the polarity of the solvent, the efficiency of the extraction is dependent upon the homogeneity of the matrix, and the mixing-ultrasonication-blending-soaking time. The mixture of sample and organic solvent is separated by filtration and washing with solvent. USE is optimized, takes > 60 min to perform, is easy to use and does not require expensive instruments. However, it requires a large volume of solvent (50^200 ml), it may require successive extractions, extracts are usually diluted and therefore require filtration, and the method is not automated [**135**, **139**].

Soxhlet extraction

Classical Soxhlet extraction is still the most common liquid-solid extraction technique that has been widely used since the late 19th century. This method is rather undemanding and cheap and can be apply to both biotic and abiotic matrixes. The size of the systems can vary, but the more common configurations use between 100 and 200 ml solvent to extract between 20 and 200 g of sediment and 1 and 100 g of biological tissue. Extraction with non-polar solvents, such as *n*-alkanes, takes a considerable time (> 6 h) and was not as effective as polar

solvents, such as dichloromethane. Although this method is effective enough for many matrixes and target analytes, it also has some disadvantages [**134**, **135**]:

- requirement for large volumes of solvent (10-200 ml for 1-100 g of tissue);
- use of highly-purified polar and non-polar organic solvents (dichloromethane, hexane-acetone, hexane-dichloromethane);
- the long extraction time (~ 18 h) as a result of slow analyte diffusion;
- desorption from the sample matrix to the extraction fluid;
- dilution of the extracts obtained;
- generation of "dirty" extracts that require extensive clean-up;
- it cannot be automated (or with difficulties) ;

An improved extraction technique, based on the Soxhlet system, is Soxtec, which is a two-step procedure, involving a boiling and a rinsing step, which drastically reduces the total time of extraction. It has been used in several applications to extract organochlorine contaminants from solid samples [**133**].

Supercritical fluid extraction (SFE)

SFE has attracted intense interest during the past 20 years, mainly for extraction of solid samples, because it offers short extraction times and minimum use of organic solvents [**140**]. The attraction of the SFE technique is directly related to the unique properties of the supercritical fluid which has a low viscosity, high diffusion coefficients, low toxicity and low flammability, all of which are clearly superior to the organic solvents normally used. Carbon dioxide is the most common supercritical fluid to be used, since it is inexpensive and has a low critical temperature (31.3 °C) and pressure (72.2 atm, i.e. 7.3 MPa). Other less commonly used fluids include nitrous oxide, ammonia, fluoroform, methane, pentane, ethanol and dichlorofluromethane [**134**]. Fat retainers are usually introduced into the extraction thimble to achieve a fat-free extract; the most commonly used fat retainers are basic alumina, neutral alumina, Florisil® and silica [**141**]. SFE, on the other hand, suffers from operational problems with a need to optimize many parameters (especially analyte collection), problems for matrices with high water content (such as blockage of the restrictor) and the high cost of automated instruments. SFE gave results comparable to SOX for PCB congeners using a reference material (SRM 2974) in a freeze-dried mussel tissue [**142**]. SFE employing CO_2 as the extraction solvent has been commonly used for determination of PCBs, OCPs, and PAHs in an array of various samples incl. soils, sediments [**143**, **144**], and biota tissues [**145, 146**].

Accelerated solvent extraction (ASE)

This technique, also known as pressurized fluid extraction (PFE) or pressurized liquid extraction (PLE), is one of the most recent solid sample extraction methods. It uses organic solvent or a mixture of solvents and increased pressures and temperatures to speed up extraction of POPs from environmental matrices. The use of higher temperature increases the ability of solvent to solubilize the analyte, decreases the viscosity of liquid solvents, thus allowing better penetration of the solvent into the matrix and promotes 'wetting' of the matrix particles so as to achieve contaminant recovery [**147**].

Tab. 11: Comparison of traditional and recent extraction techniques [**136**]

	Extraction technique					
	MAE	**FMASE**	**PLE (ASE)**	**SFE**	**Soxhlet**	**Sonication**
Brief description	Sample is immersed in a microwave-absorbing solvent in a closed vessel and irradiated with microwave energy.	Sample is immersed in a microwave-absorbing solvent in an open vessel and irradiated with microwave energy.	Sample and solvent are heated and pressurized in an extraction vessel. When the extraction is finished, the extract is automatically transferred into a vial.	Sample is loaded in a high pressure vessel and extracted with supercritical fluid (most commonly CO_2, 150-450 bar and 40-150 °C). The analytes are collected in a small volume of solvent or onto a solid-phase trap, which is rinsed with solvent in a subseq. step	Sample is placed in a glass fibre thimble and, by using a Soxhlet extractor, the sample is repeatedly percolated with condensed vapours of the solvent.	Sample is immersed in solvent in a vessel and placed in an ultrasonication bath.
Extraction time	3–30 min	10–60 min	5–30 min	10–60 min	3–48 hrs	10–60 min
Sample size	1–10 g	1–30 g	1–30 g	1–5 g	1–30 g	1–30 g
Solvent usage	10–40 ml	10–150 ml	10–100 ml	2–5 ml (solid trap) 5–20 ml (liquid trap)	100–500 ml	30–200 ml
Investment	Moderate	Moderate	High	High	Low	Low
Advantages	✓ Fast and multiple extractions ✓ Low solvent volumes ✓ Elevated temperatures	✓ Fast extractions ✓ Low solvent volumes	✓ Fast extractions ✓ Low solvent volumes	✓ Fast extractions ✓ Minimum solvent volumes	✓ No filtration required	✓ Multiple extractions
Drawbacks	☹ Extraction solvent must be able to absorb microwaves ☹ Clean-up step needed ☹ Waiting time for the vessels to cool down.	☹ Extraction solvent must be able to absorb microwaves ☹ Clean-up step needed ☹ Waiting time for the vessels to cool down.	☹ Clean-up step needed	☹ Many parameters to optimize, especially analyte collection.	☹ Long extraction times ☹ Large solvent volumes ☹ Clean-up step needed	☹ Large solvent volumes ☹ Repeated extractions may be required ☹ Clean-up step needed

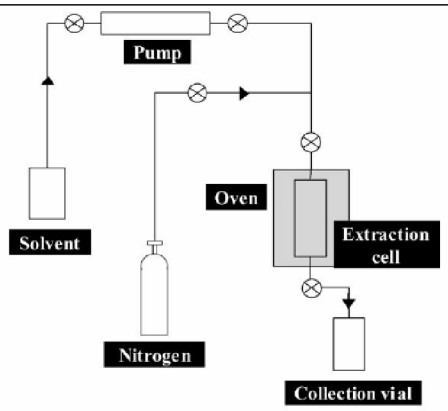


Fig. 23: ASE system scheme

The use of higher pressure facilitates the extraction of analyte from samples by improving the solvent accessibility to the analytes that are trapped in the matrix pores. By pressuring the sample cell, it is possible to keep the organic solvent in a liquid phase, even at relatively high extraction temperatures and pressures (up to 200 ºC and 20 MPa). Common ASE appliances comprise a stainless-steel extraction cell, where programmed temperature and pressure are controlled by electronic heaters and pumps (**Fig. 23**). The time of extraction step ranges from 5 to 15 min. This technique, besides low solvent consumption (hence broadly recognised as a "green" extraction technique) and extraction time needed, has some another advantages of being fully automated and having option to control pressure, temperature and solvent polarity (either pure or blend), according to the type of matrix and analytes to be extracted. The ASE processes can be carried out in both dynamic and static mode, as e.g. SFE. However, the main disadvantage of ASE is that sample clean-up is still necessary. The fat content in extract depends on the matrix fat percentage. Furthermore, it is also necessary to dehydrate the sample beforehand in order to extract the lipid from the biological tissues. Anhydrous sodium sulphate or Hydromatrix are usually used as a dehydrating agent for ASE extraction of environmental samples such as soil or animal tissue. ASE shows extraction efficiency comparable or slightly higher to that of Soxhlet for the PCB spiked organic matrix. PCB recovery from spiked matrix is dependent on the type and molecular weight of congener, and nature of matrix [**147**]. ASE has been reported to be a good method for the extraction of POPs and other halogenated compounds from environmental matrices, such as soil [**135**, **149**], plant, or house dust [**150**]. PAHs have been determined by ASE in various marine matrixes (mussels, fish, etc.) [**151**] and sediments [**152**]. However, there are still few reports, which examine the lipid extraction efficiency from biological tissues using ASE [**148**].

Microwave-assisted extraction (MAE)

MAE is a rather new extraction method put in practise in the 1980s. It is suitable for the extraction of solid matrices and can use both polar and nonpolar solvents. MAE allows rapid extraction of solutes by employing microwave energy as a source of heat, with extraction efficiency comparable to that of the classical techniques. Microwave energy (0.016 eV, 2,450 MHz), being absorbed by a solvent (acc. to its dielectric constant; the solvent must be polarised), causes molecular motion by migration of ions and rotation of dipoles. Microwave absorption results from permanent dipole reorientation caused by the electric field. As the field decreases, thermal disorder is restored resulting in the release of thermal energy. Unlike other heating techniques, where the heat penetrate through the extraction vessel, microwaves heat the entire sample simultaneously without heating the vessel; thus, the solution reaches its boiling point very rapidly, leading to a very short extraction time [**139**]. It is additionally believed that high efficiency is obtained due to a destruction of the matrix macrostructure. The major drawback of MAE seems to be the lack of selectivity which results in the co-extraction of significant interfering compounds.

The application of microwave energy to the sample may be performed using two technologies:

- closed vessels under controlled pressure and temperature, a process referred to as **pressurised** MAE (**PMAE**)
- open vessel under atmospheric pressure, referred to as **focused** MAE (**FMAE**).

The use of closed vessels allows an operating temperature higher than the boiling point of the solvent, further reducing extraction time [**153**]. On the other hand, the main drawbacks of such a system are loss of more volatile solutes if the temperature inside the vessel rises rapidly, and the vessels need to be cooled to room temperature after extraction and before they can be opened, thus increasing the overall extraction time [**139**]. In open systems, as extractions proceed under atmospheric pressure, the maximum possible temperature is determined by the boiling point of the solvent. Sample heating is carried out homogeneously and efficiently. In conclusion, compared to e.g. SFE, investment cost is nevertheless smaller in case of MAE (or ASE) and optimisation of the few parameters can easily be carried out. At present, MAE and ASE are preferred to a less versatile SFE and there are e.g. multi-vessel MAE systems commercially available, drastically increasing the sample throughput.

Solid-phase (micro)extraction (SP(M)E)

Today, solid-phase extraction (SPE) is widely accepted as an alternative extraction/cleanup method to liquid-liquid extraction (LLE) for determination of various pollutants in liquid samples. SPE is more advantageous than LLE because of decreased sample-preparation time, solvent usage, and improved sensitivity and repeatability. However, drawbacks such as clogging of the SPE cartridge and breakthrough problems may occur [**140**]. SPME is an easily-automated, simple, one-step, rapid and solvent-free extraction method. It was introduced in the early 1990s, and is mainly suitable for aqueous matrices. This technique does not suffer from the plugging or channelling problems encountered with SPE. In addition, it completely eliminates organic solvents. SPME also involves fewer steps and less sample handling. The method is based on partitioning the analytes between the water phase and the organic coating (direct extraction), or between the gas phase above the sample and the SPME fibre (headspace (HS) extraction). Direct extraction is the method of choice for clean samples, whereas HS extraction is more suited to dirty liquid samples, or to samples that may be quickly photodegraded. By performing HS extraction, a single fibre could be used 50-100 times. After extraction is completed, the coated fibre is introduced into a GC injection port, whereas the analytes are released in 1-10 min by thermal desorption.

Both SPE and especially SPME are widely used for determination of POPs in various samples including human blood serum [**154**], water [**155, 156**] or air [**157**].

2.3.3. Extract clean-up (co-extract removal)

Whatever technique is used for extraction, various matrix components such as lipids, carotenoids, pigments and resins are frequently present in the extract and must be eliminated to permit a more definitive identification and quantification of lower levels of analytes and to minimise deterioration of chromatographic performance. Thus, the removal of co-extracted matrix components is critical and different clean-up procedures have been developed to minimise their negative effects. Moreover, the clean-up step is usually necessary to remove not only the bulk of the co-extracted material, but also those compounds closely related to the analytes that could potentially interfere in the final determination. If not removed from the extract, those co-extracts can affect final chromatographic analysis in several ways: (I) lipids may contaminate injector cell or column of gas chromatograph, (II) some other compounds (PCNs, toxaphenes, etc.) may co-elute with target analytes and cause false results, (III) co-extracted material may cause peak interferences, noisy baseline or negative peaks when electron capture detector (ECD) is employed. All measurable traces of lipids should be removed especially prior to the final GC detection and preferably prior to group separation on the PYE or PGC-HPLC, because of irreversible damages to the column phase [**134**].

The determination of lipophilic organochlorine contaminants in biological samples is often linked with the lipid content determination. Owing to the fact that the POP tissue levels highly depend upon the lipid content of the material several methods are applied to determine the total organic carbon (TOC) content in the sample so that the interspecies differences (fat content versus POP levels in tissues; seasonal factors, etc.) can be taken into account. Moreover, detailed knowledge of lipid content facilitates the clean-up step optimisation. In general, lipids are extracted from the tissue using a blend of solvents (esp. chloroform/methanol) and after centrifugation and evaporation the lipid content is determined gravimetrically (Bligh-Dyer's method, [**207**]), or the lipids are extracted from the sample by using wet oxidation (Walklay-Black's method, [**158**]).

Two different approaches are employed for the clean-up of lipids: non-destructive (GPC, adsorption columns, or dialysis) or destructive ones (saponification, oxidative dehydration). Let us revise the most common strategies for extract clean-up. Brief characteristics of the various PCB lipid-removal techniques are shown in **Table 12**.

Tab. 12: Comparison of lipid removal techniques [**134**]

Technique	**Requirement**							
	Lipid removal	**Destruction of CBs**	**Destruction of other components**	**Recovery**	**Amount of time spent**	**Amount of work invested**	**Batch variability**	**Automation**
GPC	2 steps necessary	NO	NO	Good	High	High	NO	Easy
Florisil alum. silica	Low capacity	NO	NO	Good	Fair	Fair	Yes	Difficult
Dialysis	Fair	NO	NO	Good	High	Fair	NO	Difficult
Acetonitrile back-extraction	Fair	NO	NO	Good	High	High	NO	Difficult
Sulphuric acid (and or H_2SO_4/ silica)	Good	NO	OCPs	Good	Low	Fair	Negligible	Difficult
Saponification	Good	High-Cl PCBs	OCPs	Fair	Low	Fair	Negligible	Difficult

NO = not observed;

Gel-permeation chromatography (GPC)

This technique, also referred to as size exclusion chromatography (SEC), is based on molecular size separation where especially molecule sizes and shapes are crucial, but some other sorption phenomena may participate as well. GPC separation is used primarily to fractionate and remove lipidic material (>50 nm), which elute first from the column followed by the smaller molecules which include most of the organic contaminants that accumulate in biological tissue. The commonest stationary phases are styrene and divinylbenzene copolymers often modified by various functional groups (Styragel, Bio-Beads). Most laboratories use SX-3 BioBeads (200-400 mesh) in a range of column sizes (from 50×10 mm up to 600×25 mm) and solvents (hexane, chloroform or blends of solvents).

GPC cleanup has been approved by EPA for the purification of organic extracts from solid environmental samples [**160**], Suchan et al. [**161**] reported the purification of PCBs and OCPs in fish extracts by GPC on a S-X3 Biobeads column with cychlohexaneethyl acetate (1:1, v/v) as mobile phase. Similarly, the same GPC column but with chloroform as mobile phase was used for the cleanup of PAHs in spruce needles and fish tissue extracts [**162**]. GPC cleanup has shown to be suitable for the determination of trace contaminants in fish, because of approx. the same membrane permeability (up to 50 nm) [**134**].

Several key advantages of GPC, over other methods currently available for the clean-up of lipids, include unlike typical saponification or concentrated sulphuric acid treatments (**I**) it is non-destructive, (**II**) it allows handling larger masses of lipids in each sample (e.g., columns of ca. 500 × 25 mm ID can handle up to 500 mg of fat compared to adsorption columns limited to 50 mg/g of lipids [**140**]) and (**III**) GPC is more applicable than adsorption chromatography to "unknown" contaminants isolation when little information on the polarity or chemical functionality of the molecule is available. Finally, (**IV**) it can be fully automated.

One main disadvantage of the GPC system is that it is difficult to completely remove all traces of the lipids. Since the triglycerides elute prior to the smaller contaminants the "tail" of the lipid peak intrudes into the second fraction. According to Grob and Kälin [**159**] much of the tailing is caused by lipids trapped in the injection port and the connecting tubing. Even a 0.01% carry-over from 1 g of lipid leaves an unacceptably high level of co-extracts in the extract which can affect group and final chromatographic separation and quantification. Therefore, further clean-up steps are often necessary including adsorption chromatography or sulphuric acid treatment.

Classical adsorption chromatography

The method involves passing extracts through several adsorbent columns prepared in the laboratory or through solid-phase extraction cartridges. Adsorption chromatography can discriminate between the target compounds and the matrix components to a degree that depends on the selectivity of the sorbent(s) used. Moreover, it is used in group separation schemes to isolate the CBs from OCPs and other trace organics. Alumina, silica gel and Florisil columns in different mesh sizes, levels of activity and column sizes (either separately or in combination) are widely used [**163**, **134**, **164**]. However, sometimes, an alkaline treatment (saponification) or a treatment with sulphuric acid is necessary prior to, or in conjunction with, adsorption columns to remove the bulk of co-extracted lipids, and it may be necessary to use more than one column (adsorbent) to obtain adequate clean-up and/or fractionation of sample extracts. This method's drawbacks include limited column capacity

(alumina columns of 10-20 g have a fat capacity of ~250 mg, which may not be enough to remove large quantities of lipids [**138**]), its laboriousness and the demand for new validation of any column and/or sorbent changes. Nevertheless, a commercially available automated clean-up multi-column system which can clean-up ten extracted samples containing up to 1 g of lipids in less than 2 hrs has been evaluated for the determination of PBDEs, PCBs, PCDDs and PCDFs in fortified beef fat as quality control samples. [**165**].

Dialysis

Polyethylene (PE) film of pore size ca. 50 μm can be used to dialyse the organic extract to isolate the PCBs from the fat. The PE membrane has a molecular mass cut-off of ca. 50 nm, recoveries approaching 95% has been reported [**167**]. To remove all impurities, the membrane has to be conditioned by solvent before used. It can be used in the analysis of numerous POPs of different concentrations, molecular shapes and sizes.

Although this method is simple, effective and can dialyze more than 20 g of lipid as a single sample, it requires a large volume of solvent to be used, and it is time-consuming (up to days). Lipids also co-extract during the procedure up to 1-2% of the total lipid. [**166**].

Saponification

It falls into destructive clean-up methods and proceeds from the hydrolysis of nonpolar triacylglycerol present in the extract sample. Polar degradation products remain in the sample whereas nonpolar compounds are isolated by a nonpolar solvent. This approach is suitable for analytes resistant to strong bases. Lipids are saponified by heating the extract in a small volume of solvent with 20% ethanolic KOH solution at ca. 70 ºC for 30 min [**168**]. However, Van der Valk and Dao found that PCB 180 was partially degraded during the saponification of sewage sludge when temperatures were above 70 °C for more than 30 min [**169**]. Metal particles in sediments may possibly catalyse this dechlorination. Some more chlorinated PCB congeners (CBs 170, 194, 195, 201,205, 206, 207 and 209) were found to degrade at 60°C over 30-90 min (lipids were completely eliminated in 30 min.) [**170**]. The degradation of PCB 180 may lead to non-*ortho* CB 169, bringing about absolutely incorrect analytical results.

Oxidative dehydration

The main alternative, destructive clean-up method to saponification is oxidative dehydration with concentrated sulphuric acid. Concentrated sulphuric acid is mixed with the lipid extract (which is usually dissolved in pentane, hexane or hexane/dichloromethane) and vigorously stirred for a few minutes; the clean-up procedure is repeated 4-6 times; this is followed by separation of the organic layer in a funnel; the solution is next evaporated for dryness, finally, the cleaned extract is redissolved in an organic solvent for analysis [**171**]. Alternatively, H_2SO_4 is added to silica and the sample is eluted from the column. This technique is fast, efficient and can remove large quantities of lipid (20 g or more; a column of 50 g of SiO_2-H_2SO_4 can remove 10 g of lipid). However, this method in not suitable for labile compounds (aldrin, endrin, etc.). Degradation of CBs has also been reported [**134**]. Owing to potential losses of chlorine atoms, destructive clean-up techniques are not too preferred for chlorinated POPs at present [**208**].

Integrated extraction and clean-up

So far, few publications have been published dealing with on-line combined extraction and clean-up procedures based on SFE or ASE treatments [**164**]. The suitability of integrating the clean-up step into extraction techniques by resorting to the use of sorbents in the extraction cell which would retain the matrix components (trapping sorbents) have been examined. For instance, Björklund et al. [**172**] assayed for five different fat retainers, including Florisil, basic alumina, neutral alumina, acidic alumina and sulphuric acid-impregnated silica in order to clean-up PCBs in fish using ASE and the use of sulphuric acid-impregnated silica provided the cleanest PCBs extracts. Sulphuric acid-impregnated silica as fat retainer in ASE was also successfully used for the determination of PCBs in several fat containing matrices. In another approach, the ASE cell was filled up with aluminium oxide, silica gel and magnesium sulphate for the extraction and purification of PAHs in blue mussel and salmon [**174**], but a further purification by GPC was required. ASE extraction of PCBs from biota tissues by on-line clean-up with sorbents such as Florisil, silica gel, alumina, or 2,3-dihydroxypropoxy-propyl (Diol) in the extraction cell has been reported by Gomez-Ariza et al. [**173**].

2.3.4. Fractionation/group separation

Since there are many GC columns available on the market and capable of sufficient resolution for exact quantification of major organochlorines, normally, when only major PCB congeners or important OCPs are to be determined (excl. critical PCB pairs, which can be separated using different capillary and/or stationary phase), it is not necessary make a series of group separations prior to the final resolution of analytes by high resolution gas chromatography. Pre-separation of PCBs is required prior to analysis in order to separate important and toxic non-*ortho* and mono-*ortho* PCBs present at substantially lower concentrations compared with remaining PCBs and other POPs (with the exception of mono-*ortho* PCB 118, and to a minor extent PCB 105), since the range of analyte concentrations is normally too large for all compounds to be measured without additional dilution or concentration, and some of key PCBs are not resolved on a single GC column, regardless of the column phase [**134**]. The cleaned-up extract may contain other organohalogens such as OCPs, polychlorinated naphthalenes (PCNs), PCDDs, PCDFs, polychlorinated camphenes (PCCs) (Toxaphene) as well as the PCBs [**175**]. For that reason, it is quite impossible (except using multidimensional chromatography) to determine all compounds present in one fraction by using common single column configuration without adequate dilution or concentration, because there is a significant concentration gap between individual POPs.

The methods used for dividing PCBs into individual separated groups utilise spatial planarity of these molecules. Several techniques including classical adsorption materials (alumina, silica gel or Florisil), charcoal, porous graphitic carbon or a special silica-bonded PYE column have been widely described in the literature. Comparison of fractionation techniques is in **Table 13.**

Tab. 13: Comparison of fractionation techniques [**134**]

	Requirement				
	Separation	**Batch variability**	**Lipid sensitivity**	**Interference from column material**	**Automation**
Gravity columns of alumina silica or Florisil	Difficult	High	Low	Low	Difficult
Charcoal	Fair	High	Low	High	Difficult
Porous graphitic carbon-HPLC	Good	Low	High	Low	Easy
Pyrenyl silica-HPLC	Good	Low	Very high	Very low	Easy

Adsorption chromatography

Classical sorbents like Florisil or alumina have been used for fractionation, because they are relatively cheap, available, and hence often employed. Their drawbacks include batch variability, hygroscopic properties (need for standardisation), and demand for activation/partial deactivation after reaching required adsorption effect. Normally, these absorbents are adjusted to a specific activity with water in order to remove co-extracted materials. These adsorption methods are suitable for the separation of the planar PCBs from the other *ortho*-PCBs only and the matrix must be thoroughly cleaned-up prior to this separation being made. Normal lipid removal cannot be made simultaneously on the same column. Moreover, a gradient elution (hexan-DCM, etc.) is required to obtain the mono-*ortho*, non-*ortho*, and PCDDs/PCDFs fractions. Storr-Hansen and Cederberg [**177**] studied various adsorbents heated at 450°C to fully activate the material which was used without further deactivation. A mixture of PCBs, including the non-*ortho* congeners, was reapplied to a column containing activated basic alumina and eluted with hexane. Firstly, the elution volume for the bulk of the PCBs increased from around 10-20 ml (deactivated form) to 150 ml (fully activated form), and secondly, the planar congeners, PCBs 37, 81, 77, 126 and 169 were only partially eluted after some 300 ml. Similar studies with active Florisil gave approximately the same pattern of retention of the planar PCBs, but with a smaller elution volume. It is supposed that highly activated silica is very hygroscopic (the adsorbent will become deactivated to at least 1% due to the surrounding water vapour in the laboratory atmosphere). When the alumina and Florisil were deactivated to >3% with water the separation between the planar and non-planar PCBs disappeared and all PCBs were eluted with 20-30 ml hexane.

After comparing absorption abilities of various adsorbents for a separation of planar PCBs and 2,3,7,8-TCDD/TCDF, a correlation between the sorption of analytes and their molecular structures and the π-electron cloud localisation was found. The best separation and recoveries were achieved using 6 g of Florisil activated at 450 °C over 24 h [**209**].

The separation of the *ortho*-, non-*ortho* PCBs, and PCDDs/PCDFs for routine use was described by using 25 g of alumina. *Ortho* PCBs were eluted with 150 ml of hexan-DCM (48:2), non-*ortho* PCBs with 200 ml of hexan-DCM (9:1), and PCDDs/PCDFs with 200 ml of hexan-DCM (1:1) [**210**].

Activated carbon, charcoal

Activated carbon has been used extensively to separate the non-*ortho* and the mono-*ortho* PCBs from the remaining congeners [**178**]. The attraction of using activated charcoal is that it has a high affinity for organics even at the ultra-trace level, it is inexpensive, readily available and easy to use. On the other hand, various interfering impurities (high blank values), and higher retention times of target analytes (i.e. more solvent needed for elution; possibly irreversible sorption) are the main drawbacks. Moreover, activated carbon also has a limited application for a small number of samples and needs to be prewashed thoroughly before used.

An extensive and thorough comparison of six activated charcoals made by Kannan et al. [**179**] showed that the charcoals tested were not able to completely separate the non-*ortho* PCBs from the dominant *ortho* PCBs. In particular the co-elution of PCB 110 with PCB 77, and of PCBs 129 and 178 with CB 126 was sufficient to prevent the quantification of the planar congeners. With the advantage of the MDGC it was possible to identify the problems of separation with these materials. Data for PCB 77 and PCB 126 obtained by these forms of activated charcoal, when not using MDGC, were likely to be an overestimate.

A mix of activated carbon and silica gel can be used as well. A 1:20 mix of activated AX-21 carbon and low-pressure silica gel LPS-2 was used for separation of non-*ortho* PCBs (elution of DCM-cyclohexane, 50 ml) from the planar PCBs 77, 81, 126 and 169 (elution of toluene, 40 ml). The recoveries were reported to be between 82 and 96% [**180**]. The best separation of all PCBs (60 ml of DCM-hexane, 1:3) and PCDDs/Fs (200 ml of toluene) was achieved by using 1 g of a activated carbon/silica gel mix (200 mg/g) [**211**]. A mix of activated PX-21 carbon (3.85 g) and silica gel (10 mg/g) was used for determination of non-*ortho* PCBs in fish and marine mammals: *ortho* PCBs and pesticides were eluted by 18 ml of hexane, non-*ortho* PCBs by 22.5 ml of DCM-benzene (1:1), and PCDDs/Fs by 25 ml of toluene in a reverse way [**212**].

Another approach employs an activated carbon (Carbopak C)/Celite 545 (1:1) column. After cleaning the column in situ the *ortho* PCBs are eluted with hexane (50 ml) and the non-*ortho* PCBs 37, 77, 81,126 and 169 are removed with toluene (15 ml). Despite lower consumption of solvents, coeluted PCNs with PCBs are the main disadvantage [**181**].

Alternatively, a mix of active SP-1 coal and chromosorb (0.5 g, 1:10, activated at 150 ºC overnight) was used to to separate the 2,4-*ortho* PCBs and *p,p*'-DDE with hexane-DCM, and the mono-*ortho* and non-*ortho* PCBs by eluting with toluene [**182**].

Porous graphitic carbon (HPLC-PGC)

The porous graphitic carbon (PGC) HPLC packing ordinarily contains the carbon with a surface area of 150 m^2/g and mean particle size of 7 μm, with a pore volume of 2 cm^3/g. This type of carbon is suitable for HPLC systems and its use makes fractionation more effective and faster. PCBs are divided into groups according to the numbers of chlorine atoms in the *ortho* position [**134**]. The HPLC-PGC column (50 × 4.7 mm i.d.) was used successfully for separation of OCPs and the *ortho* PCBs (hexane, 10 ml, 1 ml/min) from the non-*ortho* PCBs (hexane, 90 ml). Then, the column was backflushed with a further 200 ml of hexane to remove the PCDDs and PCDFs. The advantage of such a system is that the *ortho* and non-*ortho* PCBs can be separated using a single solvent (isocratic system) with an HPLC system being fully automated.

The same PGC 'Hypercarb' column, but with a solvent mix of DCM-cyclohexane (1:1, 2 ml/min) was employed to separate toxic PCBs in animal fat. The increase in polarity reduced the elution volume of the PCBs (60 ml for multi-*ortho*, 60 ml of toluene for the non-*ortho* PCBs) [**183**].

Reversing the flow of hexane ('backflush') after the elution of the *ortho* PCBs can further speed up the recovery of the non-*ortho* PCBs [**184**].

Two coupled HPLC columns can be used to improve the isolation of the PCBs according to their *ortho* substitution pattern. An aminopropyl C-18 column (250 × 10 mm i.d.) is coupled with a Hypercarb column (100 × 4.7 mm i.d.). After removing lipid residues, the extracts are eluted with n-hexane through the aminopropyl column to isolate aliphatic and monocyclic compounds, e.g. HCB. The second fraction containing PCDD/Fs, PCNs and PCBs, is switched to the second column. The elution of the tetra-, tri-, and di-*ortho* PCBs is carried out by using n-hexane, while the first column (now uncoupled) is being backflushed in order to remove the polyaromatics (10 ml). Subsequent step involves eluting the mono-*ortho* PCBs from the PCG column with 15 ml of DCM-hexane (1:1, 2 ml/min), then the column is being flushed by this solvent mix, and finally with a DCM-methanol mix (1:1). The last fraction containing the non-*ortho* PCBs, PCDDs, and PCDFs is isolated by backflushing with a 60 ml of toluene at 40 ºC [**185**].

Pyrenyl-silica column (HPLC-PYE)

The PYE column (2-(1-pyrenyl) ethyldimethysilylated silica, **Figure 24**) in combination with MDGC-ECD is a sensitive and selective technique for the measurement toxic non- and mono-*ortho* PCBs. It allows even heavily contaminated field samples to be analysed for toxic PCB measurement [**176**]. The column contains a spheric material with a surface area of 330 m^2/g, mean particle size of ~5 μm, and a mean pore sizes of ~11 mm. Hexane is used as a mobile phase in most cases. The PYE column can separate the *ortho*- and non-*ortho* PCBs on the basis of a spatial arrangement of their molecules, i.e. the degree of planarity and chlorination. It is able to separate structurally similar molecules with different π-electron densities resulting from the spatial configuration of both aryl rings [**213**, **214**].

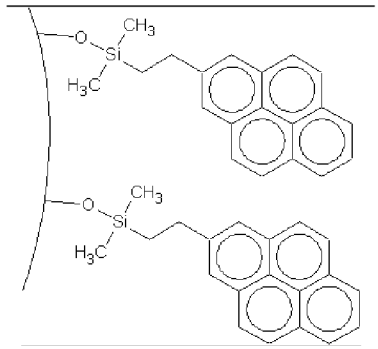


Fig. 24: PYE stationary phase

An excellent selectivity of the PYE column probably stems from a mechanism of the charge transfer – different PCB skeleton regions with particular electron densities (acceptors or donors) may induce changes in the localisation of the stationary phase π-electrons and thus condition the forming of a donor-acceptor complex. This mechanism can explain observed retention behaviour of PCB congeners:

- since chlorinated compounds are very strong electron acceptors, more chlorinated PCBs probably form strong donor-acceptor complexes with the PYE phase, because PAHs (such as pyren in the PYE phase) are electron-donating
- multi-*ortho* PCB congeners are less retarded on the stationary phase considering a steric interaction between chlorine atoms (or between the *ortho* Cl and *ortho* H atoms) which result in the rotation of the σ-bond connecting two phenyl rings – consequently,

a greater distance between the biphenyl skeleton and the stationary phase shows forming only weak donor-acceptor complexes

- Naturally, PCBs with their chlorine atoms localised in the vicinal positions (e.g. 2,3,4- or 3,4,5-) are stronger electron acceptors and hence form stronger donor-acceptor complexes with a longer retention time

The PYE column is used not only for the separation of the toxic PCBs (No 77, 126, and 161), but also for the fractionation of other key PCBs which can co-elute on the 5% phenyl methyl GC column (SE-54, CPSil 8 type). This column, therefore, has the potential to remove a number of ambiguities that sometimes occur in the final determination of the toxic PCBs where MDCG is not available.

As stated previously, the column allows individual PCBs to be separated into three fractions: the tri- and di-*ortho* PCBs (fraction I), the mono-*ortho* (fraction II) and the non-*ortho* PCBs (fraction III). The elution order of the PCBs is directly related to the degree of chlorination and inversely related to the number of *ortho* substitutions. Furthermore, the elution order varies even among those PCB isomers with the same degree of chlorination in the identical *ortho* positions, and a number of the more highly chlorinated PCBs elute in the second and third fractions. For example, the effect on retention of the *ortho*-substitution with the more highly chlorinated PCBs is seen by comparing PCBs 205, 206 and 209, where one aryl ring is fully substituted. The second ring has a 3',4',5' pattern for PCB 205, which elutes in fraction III. By adding one further *ortho* atom to the ring the elution of PCB 206 now becomes split between fractions I and II, and the fully substituted PCB 209 with the last *ortho*-chloro position substituted elutes completely in fraction I [**186**].

The separation using the PYE column has a good reproducible and repeatable within a batch calibration, and its efficiency can be maintained by regular flushing with ethyl acetate and tempering at 0 ºC. In general, cooling gives better separation characteristics and longer column-life. However, the column is susceptible to damage if the sample contains lipid residues and therefore it is essential to remove all lipophilic co-extracted materials from the sample prior to separation.

Like the activated carbon and the POC, the PCDDs/Fs can be separated from the PCBs on the PYE column. However, it is also possible to separate the PCDDs/Fs into their own fraction according to the degree of chlorination. In view of the high k' values (usually 4-150 comparing to 0.4-2.2 for PCBs) it is preferable to remove these by backflushing (especially for the hepta- and octa-chloro isomers) or by gradient elution (e.g. ethyl acetate).

The column is ideally suited for separating organics on the basis of their planar structure. The elution profile of other groups of planar compounds having AHH/EROD activity and possibly interfering with the determination of the toxic CBs was investigated. These were the PAHs, the PCNs and the PCDDs and PCDFs [**186**]. Due to the planar pyrene ring structure and strong π-π interactions, the PYE column achieves excellent separation of the PAH isomers and their derivates.

2.3.5. Final chromatographic separation

An accurate determination of (ultra)trace level toxicants in complex environmental matrices is a demanding analytical task, and thus the measurement of toxic POPs has ever been a big challenge for analysts. The modern analytical chemistry of POPs employs chromatography in most cases – either high resolution gas chromatography (HRGC) or high performance liquid chromatography (HPLC). The main reasons are obviously the low levels to be measured (parts-per-billion [ppb] to parts-per-quadrillion [ppq]) and a large number of compounds to be considered (>35 out of a total of >400).

However, it is often difficult to determine the environmental levels as the toxic compounds are present at trace concentrations. For example, information from toxicological research identified non- and mono-*ortho*-substituted PCBs as the major contributors to the total toxic effect (expressed as the TCDD equivalents, TEQ) of PCBs in organisms although they occur at much lower concentrations than the multi-*ortho* PCBs [**188**]. Even some more complex analytical techniques like multidimensional chromatography (MDGC) or a serial coupling of two columns are currently not capable of satisfactory determination of the non-*ortho* PCBs without any group pre-separation. For instance, Kannan et al. reported a 600-fold difference as a maximum between PCBs 77 and 110, beyond which a separation is only possible with MDGC [**179**]. The concentration difference between the three non-*ortho* PCBs and the major PCBs in most environmental samples varies between ca. 100 and 3000 [**189**]. For mono- and di-*ortho* PCBs a direct GC separation is possible under various conditions. Some separation techniques for different groups of congeners are listed in the **Table 14** [**134**].

Tab. 14: Separation techniques for different groups of congeners [**134**]

Method	Group / PCB No.		
	Major mono-*ortho* **105, 118, 156**	**Minor mono-*ortho*** **114, 123, 157, 167, 189**	**Non-*ortho*** **77, 126, 169**
Single-column GC without pre-separation	Possible with care	Difficult	Currently not possible
MDGC	Easy	Easy	Difficult
Single column GC with pre-separation on:			
Adsorption charcoal	Easy	Difficult	Difficult
PGC or PYE-HPLC	Easy	Easy	Easy

Every GC process includes sample injection, column separation, and a detection of eluted compounds. *Splitless* or *on-column* techniques are commonly used for injecting samples on capillary columns. The *splitless* injection is the most frequently used technique for the detection of OCPs and PCBs, however, due to the broad range of individual PCB boiling points a strong discrimination effects can occur. For the same reason, *split* injection is not recommended as well. *On-column* injection may be used as an alternative, in particular for more labile compounds, which could be degraded in the hot injector. As for e.g. PAHs, cold *on-column* injection is preferred, as this improves the resolution obtained for the first-eluting, low MW compounds, and reduces discrimination against the high MW compounds which is difficult to avoid entirely when using *splitless* injection. Large-volume injectors (LVI) are

becoming more popular, in particular because e.g. OCP concentrations in some environments (oceans) are tending to decrease. Disadvantages of LVI, as well as of the *on-column* injection, are the stronger influence of solvent-impurities in the chromatogram and contamination of the column and detector [**190**].

Single-column GC

Prior to the introduction of MDGC, one-dimensional GC (1-D GC) was the mainstay determination method, and in many laboratories, this is still preferred or available. The availability of both electron capture negative ion chemical ionization mass spectrometry (ECNI-MS) and electron capture detectors (ECD), which is very sensitive to halogens, make such approaches attractive but not always the most appropriate.

The GC separation efficiency is dependent on the following parameters [**134**]:

- **Carrier gas:** Hydrogen is preferred as a carrier gas due to its diffusivity. It offers a good resolution, even at a higher gas velocity, since the height equivalent to a theoretical plate (HETP) is relatively unaffected by the flow-rate above the optimum [**193**]. The resolution of both helium and nitrogen declines as the gas velocity is increased. Helium may be used as an alternative to hydrogen, but the extremely high pressures with very narrow columns of <0.15 mm I.D. creates practical difficulties [**192**]. Helium may be used for columns with internal diameters >0.20 mm although the resolution obtained will be less than when hydrogen is used. Nitrogen is not suitable for use in capillary GC because it drastically reduces the column efficiency, although it has been used for some MDGC applications [**191**].
- **Column parameters:** The column dimensions are not very critical for the determination of the non-*ortho* PCBs, provided that a pre-separation has been made. The three PCBs, 77, 126 and 169 and the internal standard may easily be separated, particularly where ^{13}C-labelled standards are used. This can easily be obtained on a relatively short (ca. 25-30 m), medium bore (0.20-0.25 mm) column. If, however, there is any doubt concerning the presence of other PCBs or other planar compounds in the same fraction, then the problem can be resolved by using columns with a length of 50 m and 0.25 mm i.d.. These minimum column dimensions have been determined as a result from several inter-laboratory studies on mono- and di-*ortho* PCBs [**134**]. A reduction of the internal diameter down to 0.15 mm will further improve the separation of the CBs [**192**]. A film thickness of 0.30 μm is recommended in order to enable a separation of the early eluting CBs such as CB 28 and CB 31, particularly on medium-polar columns. A thinner film thickness may be used on apolar columns to shorten the analysis time. The film thickness of only 0.1 μm is sufficient for the analysis of the non-*ortho* PCBs.
- **Stationary phases:** Both polar and non-polar GC columns can be used for the determination of the OCPs, although some OCPs may show a tendency for adsorption to polar columns. However, no one-column phase can resolve all PCB congeners with a single injection. The first complete set of retention time data for all 209 congeners (187 PCB pairs resolved, 11 co-eluted) was reported in 1984 by Mullin et al. [**194**] for the SE-54 stationary phase (50 m × 0.20 mm, 5%diphenyl-l% vinyldimethylsiloxane). However, additional GC retention data has become available for a number of CBs on different columns in recent years.

The overview of selected studies dealing with the analytics of PCBs using various stationary phases and columns is listed in the **Table 15**.

Tab. 15: The overview of important studies on capillary columns/stationary phases used for the GC determination of PCBs [**134**, updated]

Year	Author(s)	Study objectives
1988	Fischer [**215**]	179 PCBs separated on **SB Octyl 50** (50 m × 0.20 mm × 0.25 μm, 50%n-octyl-50%methylpolysiloxan)
1992	De Boer [**174**]	Retention times of 51 CBs on seven narrow bore columns, CPSil 8, CPSil 12, CPSil 19, CPSil 88 and C18 from Chrompack, SB Smectic from Lee Scientific, and the FFAP from Hewlett-Packard.
1995	Larsen [**216**]	A comparison of 11 stationary phases, **HT-8** column found to be the most suitable for the separation of PCBs without interferences
1997	Frame [**10**]	A collaborative study of 209 PCB congeners and 6 Aroclors on 20 different GC columns and on 27 different GC-ECD/ GC-MS systems
1998	Vetter [**196**]	Retention times of 209 PCBs determined by GC-MS on a newly developed **Thermocap A** phase (50% methyl–phenyl polysiloxane)
2004	Focant et al [**195**]	The separation of the 209 PCB congeners by using GC×GC–TOFMS. Four column combinations **DB-1**/**HT-8**, **DB-XLB**/HT-8, DB-XLB/**BPX-50**, and HT-8/BPX-50 were investigated. The **HT-8**/**BPX-50** set produced the best separation

Combined GC techniques

Up till now, no GC capillary column is available that can cope with the separation of all 209 congeners of PCBs. If PCB congeners and other compounds are not resolved, this may give an ambiguous identity or no specific identification of the individual compounds. This has led to change in column configuration to achieve more complete separations and it becomes necessary to run the sample in two columns of different polarity to obtain a second set of retention times. This is precisely why there have been several attempts to overcome the resolution limitations and to employ some "multidimensional" separation techniques including two parallel/serial-coupled columns or the multidimensional gas chromatography (MDGC).

In **parallel-coupled** GC or dual-column GC two columns are attached via a retention gap to one injector and, on the other side, each column to one detector (mostly ECD). In this way the analysis can be carried out on two different stationary phases at the same time which offers a substantial possibility of confirmation. For a reliable quantification it is essential that both columns have exactly the same dimensions otherwise discrimination may occur [**217**]. For the determination of the mono- and di-*ortho* congeners a pre-separation of PCBs and other possible compounds, which might interfere in the PCB chromatogram such as organochlorine pesticides (including chlordane and toxaphene) and flame retardants (brominated biphenyls and diphenylether), is, however, recommended.

Serial-coupled columns in a GC-ECD system were used for separation of PCBs by e.g. Larsen and Bowadt [**197**]. They reported an additional separation of 26 PCBs compared to single-column GC, but, on the other hand, 10 PCBs now co-eluted, whereas previously they were separated with single-column GC. A drawback of this system is the long retention times that are required. This makes the method less attractive for routine applications [**216**]. For the separation of specific PCBs (the non-*ortho* PCBs), it may, however, be useful [**134**].

Multidimensional gas chromatography (MDGC) is one of the most effective techniques used in the analysis of PCBs, for it is capable of avoiding interferences with other congeners or compounds. There are two main requirements that define the two- or multi-dimensional separation. First, the components are subjected to two or more mutually independent separation steps/mechanisms; second, once separated, the components must remain separated until the overall analysis is completed. Although GC-MS can be considered a two-dimensional (2-D) analysis procedure and is an alternative to the MDGC, isomer co-elution may still be present because GC-MS does not expand peak capacities. MDGC remains the only practical alternative to increase peak capacity.

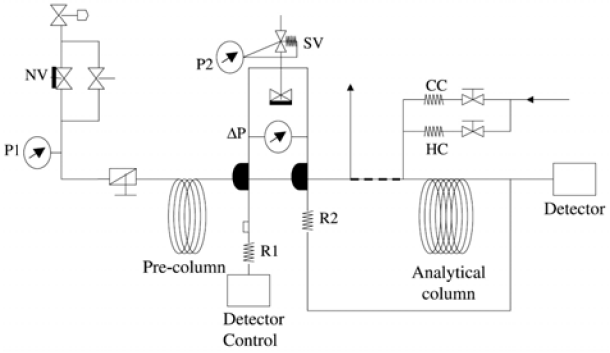


Fig. 25: Schematic diagram of a MDGC setup, based on the Siemens SiChromat II system, where: P_1, P_2, ΔP = pressures 1, 2 and differential pressure, resp.; R_1, R_2 = flow restrictors; CC = cooled coil; HC= heated coil; SV= solenoid valves; and NV= needle valves [**129**].

As can be seen in **Fig. 25**, a typical advance MDGC setup consists of dual independent ovens and detectors. The present configuration normally uses a non-polar phase on the first column (SE-54 or CPSil 8, etc.) to make the initial, well characterised separation. The sample is chromatographed on this first column to a point just prior to the elution of the unresolved peaks. The column flow is then switched into the second column of a different, usually more polar phase for the duration of the elution of these unresolved peaks only. This process is commonly referred to as *heart-cutting* and can be made with a precision of seconds. Multiple heart-cuts can be made during one run [**191**]. The resolution of this system is determined by the column(s) dimensions and the difference in separation power between the two stationary phases. The use of longer columns and smaller internal diameters improves the separation. The difference in the selectivity between the two stationary phases strongly influences the final separation; e.g., increasing the difference in polarity may be expected to improve the separation. However, the MDGC analysis system seems not to be well suited for routine, extensive analyses of complex mixtures. One of the most relevant limitations of conventional MDGC for multiresidual analysis is the fact that the increased separation power of MDGC can only be applied to a few regions of the chromatogram, rather than to the whole sample. It is extremely time-consuming to analyze many compounds in a complex sample utilizing MDGC, and the proper and accurate timing of heart-cutting is also a critical issue [**129**].

Comprehensive two-dimensional gas chromatography (GC×GC) is a relatively new technique, introduced in 1991 [**199**], and the key to GC×GC is to effect fast GC conditions for the second dimension. This method is very suitable for the analyses of complex samples, due to its increased separation potential. The setup of a typical GC×GC system involves a modulator mounted in a GC oven as depicted in **Fig. 26A**. The primary column, with dimensions similar to a typical GC experiment (e.g., 30 m×0.25 mm×0.25 μm), is connected directly to the secondary column with dimensions suitable for fast GC run (typically 1.0 m×0.1 mm×0.1 μm). Different compounds undergo different separation mechanisms, and

there are no fixed rules to the combination of column phase types as long as they provide orthogonal separation, although column sets are typically nonpolar (first dimension)/polar (second dimension). Compounds that are not resolved on the first column may be separated on the second. The analysis results then in a two-dimensional orthogonal chromatogram in which each column supplies an axis (**Fig. 27** and **28**). Owing to the fact that eluting peaks from the GC×GC analysis are very narrow (peak widths in the order of 100 to 200 ms), the sampling rate at which the detector signal is sampled should therefore be at least 100 Hz. Conventional GC detectors (FID or ECD) successfully meet this requirement, however, they do not offer any spectral information. Mass spectrometers as GC×GC detection systems could very well enhance the identification capability, but only time-of-flight (TOF)-MS is able to scan at these high scan rates (≥100 scans/s) [**200**].

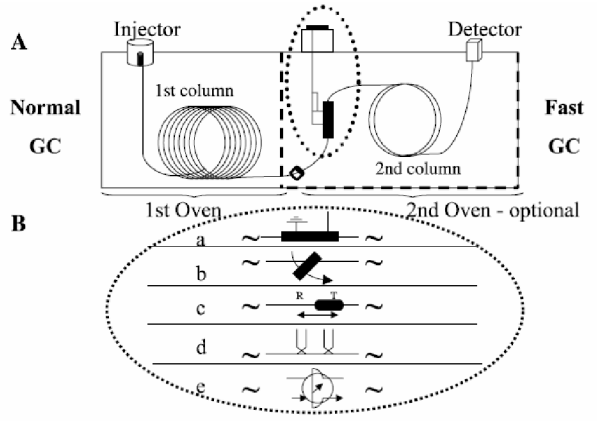


Fig. 26: Schematic of the GC×GC instrument. (B) a range of modulation mechanisms: (a) the capillary column is encased in a tube that can be pulse-heated using a supplementary electrical supply, (b) thermal sweeper, (c) longitudinally modulated cryogenic system (LMCS), (d) a dual cryogenic jet modulator, and (e) switching valve system [**129**].

The papers published specifically on environmental toxicological analysis employing this technique are not many but are increasing as chromatographers are gradually introduced to the advantages of this powerful technique.

GC detectors

The detection of eluting compounds is the final stage in a GC analysis. There are two main detectors used mostly for the analysis of chlorinated pollutants in the environmental samples: the electron-capture detector (ECD) and the mass spectrometer (MS).

ECD is the most utilized detection method for PCBs and OCPs because of its sensitivity (absolute detection limits of ca. 100 fg) and selectivity towards halogenated hydrocarbons. However, other compounds like phthalate esters or lipids may interfere and thus affect final identification of peaks. The samples injected must be hence cleaned-up thoroughly. However, it has non-linear response behaviour across a relatively narrow range (1-2 orders of magnitude), and "one-point calibration" is thus not suitable. To solve this problem of poor linearity, it is recommended to work with multi-level calibration (five or six points) which is more effective and more precise [**201**]. It is necessary for the calibration curve to be within ±5% of the true detector response. To maintain the correct level of stability of the calibration the ECD must be optimised at the correct operating temperature and be thermally stable. Normally, the higher detector temperature (320-340°C) will be sufficient, but when contamination increases the baseline signal of the detector it can normally be cleaned in situ by replacing the make-up gas with hydrogen at a temperature of ca. 400°C for 30 min. Lower flow-rates for the make-up gas (mainly nitrogen) can give a substantial increase in sensitivity, however, the response becomes increasingly non-linear at these low flows [**134**]. Since the late 1990s an improved μ-ECD has been commercially available, which has a very small cell volume and therefore yields both a higher sensitivity and a faster response [**202**].

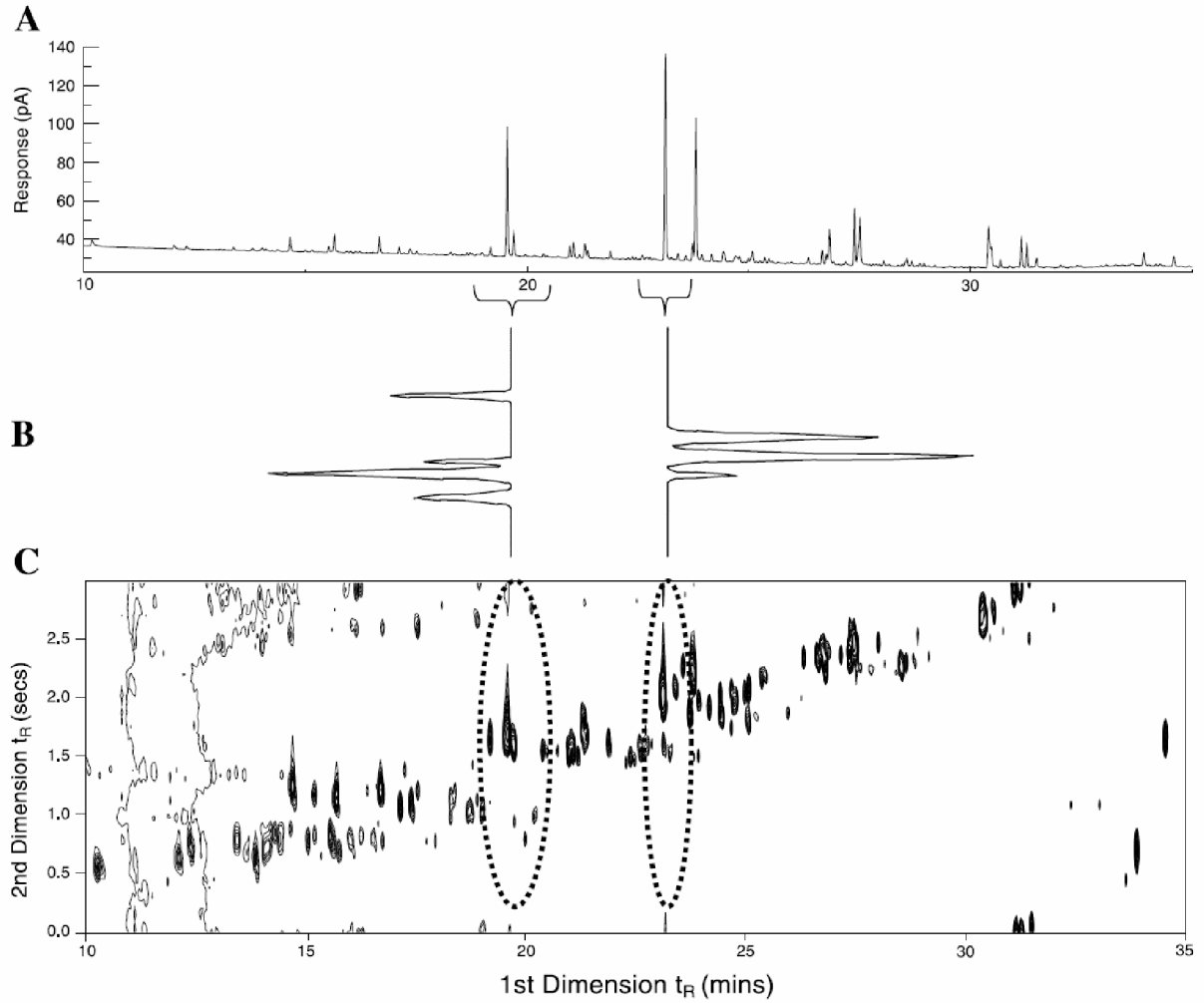


Fig. 27: (A) Chromatogram of a soil extract contaminated with PAHs. (B) Simulated heart-cut events of the soil extract illustrating that peaks unresolved in a single-column gas chromatography can be separated using the laborious and time-consuming MDGC technique. (C) Contour plot presentation of the same soil extract using the GC×GC technique. Peaks overlapping in the first dimension but separated in the second dimension were presented in the contour plot. The increase in resolution for the components by both the MDGC and the GC×GC technique can be clearly seen here. For MDGC, the resolution for the components increases only when specific heart-cut events are programmed. This requires a very accurate time frame in order to heart-cut the specific components. On the other hand, the GC×GC technique maximizes the resolution of the components throughout the entire chromatographic run [**129**].

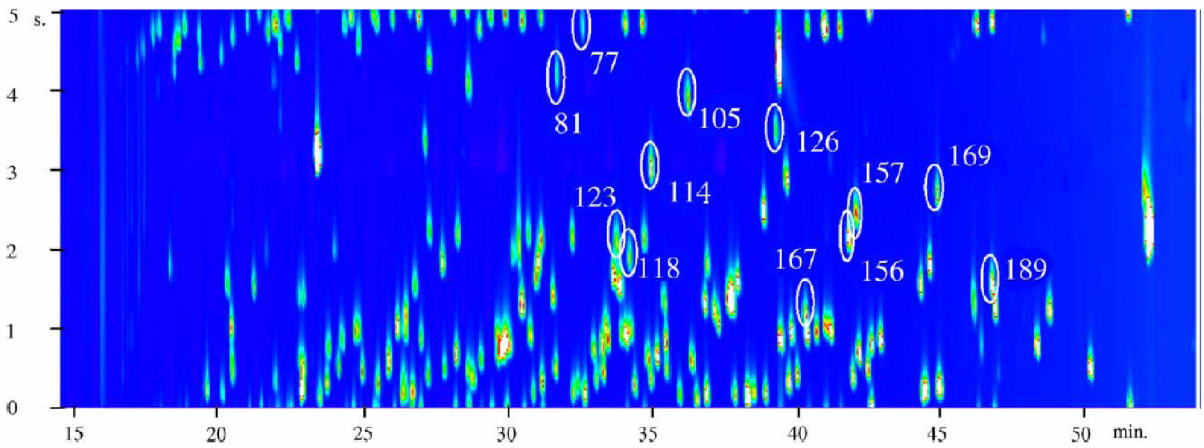


Fig. 28: GC×GC-μECD contour plot of the 209 PCB congener mixture with DB-XLB (60m×0.18mm×0.18μm) × LC-50 (2m×0.15mm×0.15μm) column combination [**187**].

Mass spectrometry (MS) is another detection technique suitable for the determination of various POP compounds which offers not only an adequate sensitivity and selectivity, but also the confirmation of the results. MS presents an important approach to the determination of (ultra)trace contaminants, becoming more and more popular. Both electron impact (EI, ionization energy of ca. 70 eV) for high resolution MS (HRMS) and electron capture negative ion (ECNI) for low resolution MS (LRMS) have been used as ionisation methods in low resolution GC–MS for OCP and PCB detection where EI is the more selective method. ECNI is more sensitive, but only for molecules which contain five or more chlorine atoms [**203**]. HRMS solution offers high detection selectivity and thus a large degree of the certainty of analyte identification, even as for co-eluted compounds. Unlike LRMS (detection limits of pg/kg), HRMS generally has higher detection limits (ng per kg) for the planar PCBs [**218**].

Different mass analysers have been used for the separation of generated ions. The choice usually depends on the mass range, resolution, sensitivity, scanning rate, and the price of an analyser.

- **Quadrupole** (scanning analyser) consists of four circular silica bars arranged symmetrically towards the longitudinal axis with a counter connection to a common potential. Ions entering quadrupole oscillate among electrodes with reverse polarities. Those ions of a specific mass stabilise their oscillation depending on the value of radiofrequency whereas other ions oscillating with increasing amplitude are being captured by the quadrupole bars. Two different modes can be theoretically employed for the scanning and quantification of ions. A SCAN mode enables all produced ions to be scanned – usually with the *m/z* ratio in range of 50-550. In a SIM mode, only the ions selected in advance and intended for quantification are monitored. The SCAN mode has lower sensitivity and is used mainly for a simple identification of compounds, while the SIM mode is more sensitive; however, the full-scan information is lost.
- An **ion trap** (ITD, scanning analyser) is, in effect, a three-dimensional quadrupole. The operation of the ion trap differs from other MS techniques, especially in the ionisation process, where the ions are trapped electronically, i.e. spatially focused. After the ionisation/trapping process, ions are made unstable according to their mass, and the destabilised ions are transferred to the electron multiplier detector outside the trap itself. With this technique, it is possible to obtain a full scan spectrum at low concentrations, for PCBs in the low-ppb range. Besides the *full SCAN* and the SIM modes, the ITD also runs in a *Segment SCAN* and MS/MS (potentially in MS^n) modes. The *Segment SCAN* is comparable to the *Full* SCAN, but it is capable of scanning more segments of different masses. In the MS/MS mode, secondary (or daughter) fragments are generated by ionisation of the original ones. The trap is able to retain the ions of a narrow mass range. However, MS/MS mode needs many parameters to be optimised when intended for a complex mixture analysis.
- Both **time-of-flight** (non-scanning) analyser (TOF) and **ion cyclotron** offer a high scanning rate which is suitable not only for classic but also for very fast chromatographic separations (GC×GC). However, acquisition costs are their main drawback. As for the TOF analyser, the separation of ions emitted from the ion source is based on the time of a segment flight through the analyser. This flight time is directly proportional to the square root of the ion *m/z* ration and ranges in milliseconds. TOF can operate either in *high speed* or in *high resolution* configuration. However, GC–TOF-MS has relatively poor instrumental limits of detection (LODs), compared with e.g. HRMS.

One of the main drawbacks of the determination of PCBs using MS detection is the similarity of mass spectra of some equally chlorinated PCB congeners (e.g. PCB 138 and 163) [**219**]. The negative-ion chemical ionisation (NCI) technique is commonly used for determination of PCDDs, PCDFs, and the non-*ortho* PCBs. GC coupled to ^{13}C-labelled isotope dilution (ID) sector high-resolution mass spectrometry (HRMS) is used for accurate identification and measurement of PCDD/Fs and PCBs in cleaned extracts [**204**].

High performance liquid chromatography (HPLC)

HPLC has also been used for e.g. the semi-quantitative detection of PCBs, because it has been demonstrated that these compounds absorb UV well enough at short wavelengths [**205**]. However, HPLC is mainly employed in the analysis of polycyclic aromatic hydrocarbons (PAHs), generally with fluorescence detection. LC is inherently more suited to the analysis of high m.w. PAH than GC, due to their limited volatility, and it can provide additional selectivity and yield separations of PAH isomers, which are difficult to separate using GC; heat-sensitive compounds are not degraded during analysis; and LC can provide a useful fractionation technique for the isolation of PAH for subsequent analysis by other techniques.

The development of reversed-phase packing materials provided a unique selectivity for the separation of PAH isomers. In combination with gradient-elution techniques and fluorescence detection, this is now an established and popular method for the determination of parent PAH. Similarly, LC with ultraviolet photodiode array absorbance detection (DAD) and to a less extent mass spectrometry (MS) have been also applied to the analysis of PAHs in various matrixes. However, analysis of PAHs using GC/MS is now preferred due to its selectivity, high chromatographic resolution and low detection limits. On the other hand, LC/MS connexion could be a good alternative approach in compliance testing, screening applications and for the elimination of matrix interference when analyzing PAHs in complex matrices as sediments. The lower chromatographic efficiency of LC is overcome to some extent by the higher separation selectivity achieved by the manipulation of both stationary and mobile phases [**190**, **206**].

3. WORK OBJECTIVES

The main objectives of this thesis were as follows:

- ✓ Assessment of the transfer and accumulation of POPs in the aquatic food chains and aquatic environments
- ✓ Determination of POPs in the terrestrial environment using conifer needles as a suitable biomonitoring tool
- ✓ Assessment of current levels of organochlorine pollutants in birds of prey from the Czech Republic
- ✓ Assessment of two modern extraction techniques – Pressurised Solvent Extraction (PSE) and Microwave-assisted Extraction (MAE) – and their usefulness for the isolation of POPs from various matrices

4. EXPERIMENTAL PART

4.1. Basic Characteristics of the Experiments

4.1.1. Transfer and accumulation of selected POPs within aquatic ecosystems

This chapter includes three closely related surveys focused on the determination of POPs in aquatic ecosystems and food webs. For a complex assessment of the contamination of aquatic ecosystems with organochlorine pollutants, both abiotic (water and sediment) and biotic (fishes) samples were taken. The following three subheads bring detailed descriptions of the realised studies and of their background.

Organochlorine pollutants in the aquatic food chain of Brno Lake

Brno Lake (see **Figure 29**) has been one of the most important recreational areas of the Brno city since its construction in the late 1930s. The water reservoir is nearly 10 km long and its total volume nears 21 mil m^3. Nowadays it functions mainly as a water energy source, a protection against floods, and a favourite place for relaxation, fishing and water sports. Due to its position in the lower part of the Svratka River basin, the water quality in the reservoir has worsened over recent years. Using phosphate washing powders in households, fertilisers and pesticides in agriculture and various industrial synthetic chemicals like PCBs have brought about significant environmental problems, including an extensive algal bloom and the contamination of lake sediments and biota, which affect particularly the recreational function of the lake.

In order to assess the present degree of the Brno Lake contamination with organochlorine pollutants, samples of water, bottom sediments, phytoplankton, and selected fish species were collected during 2007. Brief characteristics of the investigated fishes are presented in the following paragraphs (fish pictures and descriptions from [**220-222**]). The sampling sites are shown in **Fig. 29**, the list of the fishes caught is in **Table 16.**

The Asp *(Aspius aspius),* in Czech "Bolen dravý", is a European freshwater fish of the *Cyprinidae* family. It is a common species inhabiting all major reservoirs, lakes and lower reaches of rivers. During the lifetime the composition of its food changes from plankton and invertebrates to small fish and terrestrial insects. Young fish prefer to live in schools. Normally asps are between 40 and 80 cm in length, with some reaching 100 cm, and can weigh up to 10 kg. Their growth rate is very changeable and dependent on profusion of food. Since asps are very active predators consuming lots of food and energy, they generally belong to the most contaminated fish species in the Czech Republic.

The Bream or **Carp Bream** *(Abramis brama),* in Czech "Cejn velký", is a common fish species of the *Cyprinidae* family, inhabiting the lower reaches of rivers and in nutrient-rich lakes and ponds with many algae and muddy bottoms. Breams live in schools near the bottom where they dig for chironomid larvae, bivalves, and gastropods. During summer season breams eat water plants and plankton as

well. Normally they are between 30 and 55 cm in length and can weigh up to 3 kg. Their growth rate is also changeable and dependent on profusion of food. The bream is a suitable fish species for the bioaccumulation monitoring too.

The Perch or **European Perch** *(Perca fluviatilis),* in Czech "Okoun říční", is a predatory species, very widespread in watercourses and particularly in deeper valley reservoirs of the North Temperate Zone. The perch represents a greedy predator hunting its prey (zooplankton, larvae, shellfish, spawn or tiny fish) in school. Its growth rate is relatively low and its common sizes are 15 to 40 cm in length and up to 2 kg in weight.

The Roach *(Rutilus rutilus),* in Czech "Plotice obecná", is a smaller species (up to 30 cm in length) of the *Cyprinidae* family, and the most common fish species in the Czech Republic, found in both flowing and still waters. Roach live in numerous schools and as an omnivore species live on various food including small molluscs, insect larvae, annelid worms, moss, algae, or surface insects. They growth rate is very rapid in ponds and roach normally grow up to an average length of 30 cm and a weight of 1 kg.

The Tench *(Tinca tinca),* in Czech "Lín obecný", is a freshwater and brackish water fish of the *Cyprinidae* family found throughout Eurasia. The tench is most often found in still waters with a clayey or muddy substrate and abundant vegetation, and it tolerates water with a low oxygen concentration. Adults feed on bottom invertebrates and aquatic insect larvae, young also feed on algae. Its growth rate is changeable depending on profusion of food and its common sizes are about 25 up to 50 cm in length and up to 3 kg in weight.

The Zander *(Sander lucioperca),* in Czech "Candát obecný", often called pike-perch, is a common and popular game fish in Europe. Zander inhabit deep, calm waters of lakes, reservoirs and and rivers, and need plenty of oxygen. Zander is, together with the pike, the most important predatory fish of Czech waters. As greedy predators they hunt small fish and are very active mainly at night. Their growth rate is relatively high and dependent on local conditions. Common sizes are 40 to 70 cm in length and up to 8 kg in weight.

Tab. 16: Analysed fishes from Brno Lake (2007)

Date	Species (number)	Length(s)	Weight(s)	Analysed tissues
25/06/2007	Perch *(Perca fluviatilis)* (n=2)	170, 180 mm	61, 67 g	Muscle
25/06/2007	Asp *(Aspius aspius)* (n=1)	500 mm	1280 g	Muscle
26/06/2007	Zander *(Sander lucioperca)* (n=1)	n/a	n/a	Muscle
11/07/2007	Bream *(Abramis brama)* (n=2)	n/a	n/a	Muscle
11/07/2007	Tench *(Tinca tinca)* (n=1)	n/a	n/a	Muscle
13/07/2007	Bream *(Abramis brama)* (n=3)	n/a	n/a	Muscle
13/07/2007	Roach *(Rutilus rutilus)* (n=1)	n/a	n/a	Muscle

* n/a – not available

An independent study concerning the contamination of Brno Lake with organochlorines in historical terms has been carried out by the Czech Geological Survey Brno, as a part of the project "*Model of sediment and organic pollutant transport bound to suspended particulate matter in the Dyje river catchment area (2007-2011).*" Several one-to-three metre deep cores were drilled into bottom sediments in the years 2006-2008 and divided into smaller segments which were analysed separately for the content of PCBs, organochlorine pesticides and total organic carbon (TOC). The locations of the cores are shown in **Fig. 29**, their basic descriptions are in **Table 17**.

Tab. 17: Deep cores in bottom sediments from Brno Lake

Date	Sample name	Locality	Total depth	Sampling technique	Number of sub-samples
Dec/2007	BP1 A,B	Veveří Castle	75, 90 cm	Corer	6+5
Dec/2007	BP2 A,B	Kozí Horka	38, 20 cm	Corer	3+2
Dec/2007	BP3 A,B	Rakovec	5, 25 cm	Corer	2+1
Dec/2007	VB07	Veverská Bítýška	210 cm	Corer	8
Apr/2008	BP4	Veveří Castle	300 cm	Drilled core	25

Organochlorine pollutants in chub (Leuciscus cephalus) from the Svratka River

The contamination of the Svratka River ecosystem with organochlorine pollutants was assessed using selected fish species suitable for environmental pollution monitoring. During August and October 2007, specimens of the European chub were sampled by workers of the Povodí Moravy, s.p., using an electrical device (photo documentation in **Annexe 1**). The fish of specific lengths and weights were taken from two different sites at the river, mutually parted by a weird, so that the fish were not supposed to migrate between them. The first sampling site was located downstream to the Brno Waste water treatment plant Modřice (WWTP Modřice), the second site was near the village of Rajhradice. Detailed information on the caught specimens is in **Table 16**, sampling sites are shown in **Figure 29**. Immediately after catching, fish were weighed and tissues (muscle, skin and viscera) were sampled. Labelled samples were placed in microtone bags and stored in a freezing box at -18 °C until analysis. Fish age and sex were determined in collaboration with the University of Veterinary and Pharmaceutical Sciences Brno.

The European Chub or simply **Chub** *(Leuciscus cephalus),* in Czech "Jelec tloušť", is a common freshwater fish of the *Cyprinidae* family. Commonly the chub lives in variously numerous schools and can be found in both slow and moderate rivers as well as still waters of various kinds. The chub is a typical omnivore living on a variety of food from algae, various insect larvae and worms to snails, fruit or even crayfish and young water birds. The chub's growth rate is relatively low (despite its voracity) and thus it is a suitable fish species for the bioaccumulation monitoring. Normally chub are between 25 and 45 cm in length, with some over 75 cm, and weigh about 0.3-1 kg (up to 2.5 kg) [**220**,**221**].

Tab. 18: Overall summary of analysed chub from the Svratka River

Date	Locality	Number	Lengths**	Weights+	Age*
19/04/2007	Rajhradice	n = 9	221-304 mm	140-380 g	4+
19/04/2007	Modřice (WWTP)	n = 10	226-266 mm	160-250 g	3+
31/10/2007	Rajhradice	n = 11	225-270 mm	105-210 g	3+
31/10/2007	Modřice (WWTP)	n = 10	215-300 mm	80-310 g	3+

* years (average value); ** min - max values

The results were tested using ANOVA in MS Excel 2003 in order to assess the differences of POPs levels among investigated fish in terms of the sampling site, sampling period or individual tissues. Hygienic limits concerning fish quoted in the Decree of the Ministry of Health of the Czech Republic No. 381/2007 Coll. and 306/2004 were used.

Organochlorine pollutants in fish from recreational fishing

Recreational fishing has become a very popular activity (not only) in the Czech Republic during past few decades. However, environmental pollution and old burdens still negatively affects hygienic quality of fish. The background levels of old and persistent organochlorine contaminants as PCBs or OCPs, on the one hand, have already reached their peaks and now are slowly declining, on the other hand, new emerging contaminants pose new risks. In order to estimate an approximate intake of chlorinated POPs from consuming freshwater fishes and to evaluate hygienic risk, nine specimens of the common carp and one specimen of the bream were caught by an amateur fisherman from different reservoirs and ponds during the year 2007 and their edible tissues were analysed for selected organochlorines. Detailed information on the specimens investigated is in **Table 19**, fishing localities are shown in **Figure 29**. Immediately after catching, fish were measured and carved, and mixed samples of edible parts (muscle tissue incl. skin) were placed in microtone bags and stored in a freezing box at -18 °C until analysis.

Hygienic limits concerning fish quoted in the Decree of the Ministry of Health of the Czech Republic No. 381/2007 Coll. and 306/2004 Coll. and the ADI value assigned by WHO were used for the risk assessment.

The Common Carp or **European Carp** *(Cyprinus carpio),* in Czech "Kapr obecný", is a very widespread freshwater species giving its name to the *Cyprinidae* family. The common carp and its sub-species are omnivores and can feed on almost anything encountered (e.g. water plants, insects, zooplankton, shellfish, even small dead fish). The carp's growth rate is very rapid in warm and fertilised ponds and they normally grow up to an average length of 40-65 cm and a weight of 1.5-5 kg (up to 20 kg). The common carp with its 87-90% of total production belongs to the main marketed fish in the Czech Republic. For this reason the quality of the marketable carp has been monitored regularly [**223**].

Tab. 19: List of analysed samples of fishes from recreational fishing

No.	Date	Species	Pond/reservoir	Length	Weight
CA 01	04/04/2007	Common carp *(Cyprinus carpio)*	Nový Pond (Sedlec)	72 cm	n/a
CA 02	29/04/2007	Common carp *(Cyprinus carpio)*	Donbas (Tovačov Lakes)	52 cm	n/a
CA 03	06/05/2007	Common carp *(Cyprinus carpio)*	Čertův pond (Čunín)	57 cm	n/a
CA 04	14/06/2007	Common carp *(Cyprinus carpio)*	Nový Pond (Sedlec)	70 cm	5.5 kg
CA 05	23/06/2007	Common carp *(Cyprinus carpio)*	Donbas (Tovačov Lakes)	55 cm	3.3 kg
CA 06	07/07/2007	Common carp *(Cyprinus carpio)*	Čertův Pond (Čunín)	49 cm	2.5 kg
BR 01	13/07/2007	Bream *(Abramis brama)*	Donbas (Tovačov Lakes)	45 cm	1.6 kg
CA 07	05/08/2007	Common carp *(Cyprinus carpio)*	Donbas (Tovačov Lakes)	52 cm	2.2 kg
CA 08	13/08/2007	Common carp *(Cyprinus carpio)*	Donbas (Tovačov Lakes)	49 cm	2.4 kg
CA 09	13/09/2007	Common carp *(Cyprinus carpio)*	Sobáčov	60 cm	2.8 kg

* n/a – not available

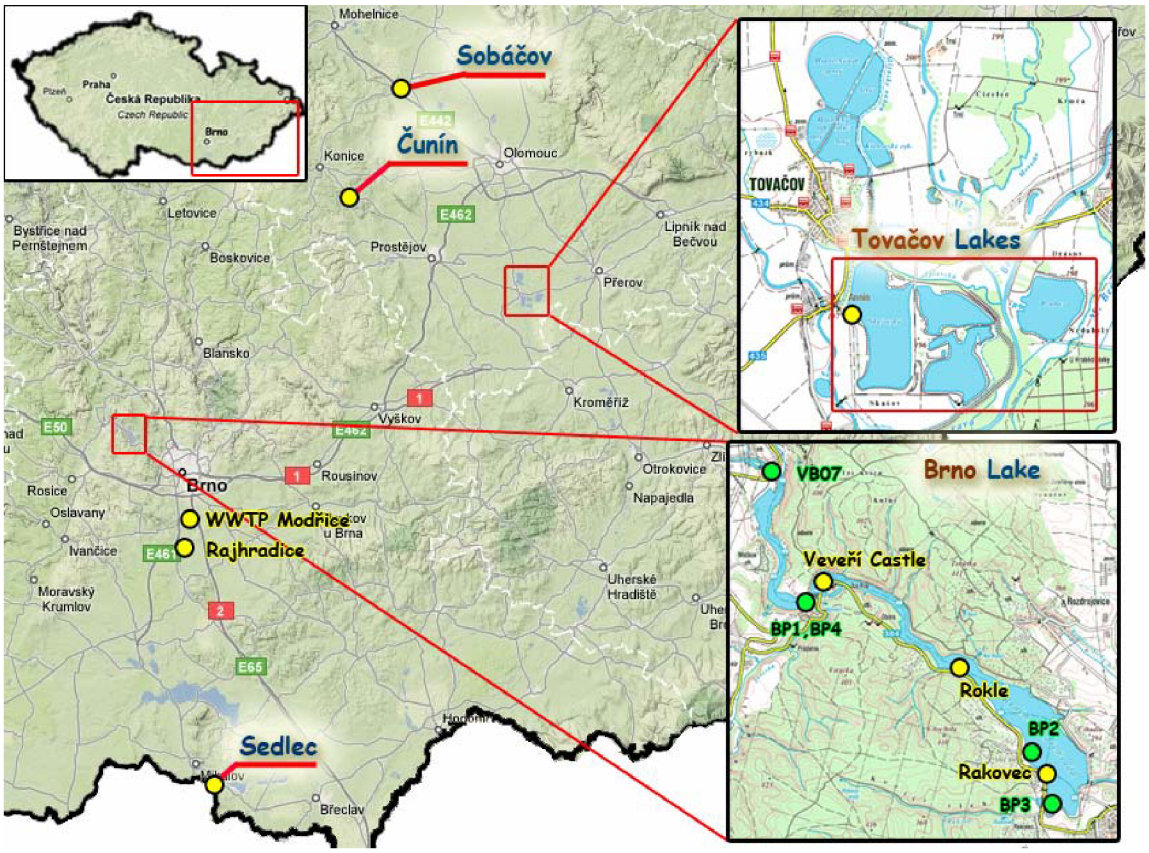


Fig. 29: Sampling localities of samples from aquatic ecosystems.

The following compounds were determined in all samples:

- PCB congeners: 28, 52, 101, 118, 138, 153, and 180
- Organochlorine pesticides and other pollutants: HCB; α-, β-, γ, and δ-HCH; *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT; octachlorostyrene (OCS)

4.1.2. Needles as a suitable tool for the monitoring of POPs in the environment

Needles of selected coniferous tree species were used for an assessment of the POPs levels in terrestrial ecosystems. Since needles have been commonly used for this purpose in several monitoring programmes (not only) in the Czech Republic, the results of this study should be well comparable with other findings. Basic information on analysed samples of needles is summarised in **Table 20**, sampling localities are marked on the map (**Figure 30**). The samplings were carried out during 2006 and 2007 and mixed samples of one- and two-year needles were taken.

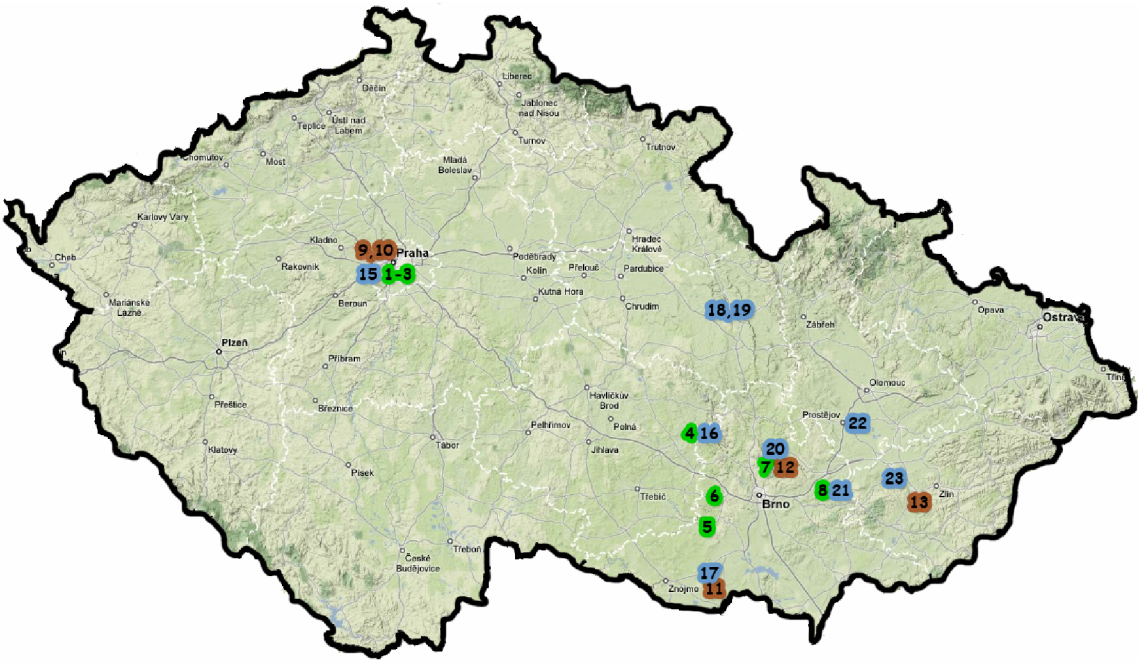


Fig. 30: Sampling localities of needles. (●) Pine, (●) Fir, (●) Blue spruce.

Tab. 20: Analysed samples of needles – description

No.	Species	Sampling locality (region)	GPS
1	**Pine**	Praha 8	50°06'44.37"N, 14°28'31.24"E
2	*(Pinus sylvestris)*	Praha 8	50°06'44.37"N, 14°28'31.24"E
3	*(Pinus strobus)*	Praha 8	50°06'44.37"N, 14°28'31.24"E
4		Radňoves (Vysočina)	49°23'19.02"N, 16°12'50.29"E
5		Moravský Krumlov (South Moravia)	49°02'56.15"N, 16°18'42.01"E
6		Zastávka (South Moravia)	49°11'16.79"N, 16°21'47.15"E
7		Vranov (South Moravia)	49°18'35.37"N, 16°36'58.12"E
8		Hlubočany (South Moravia)	49°13'51.77"N, 16°59'58.88"E
9	**Fir**	Praha 8	50°06'44.37"N, 14°28'31.24"E
10	*(Abies alba)*	Praha 8	50°06'44.37"N, 14°28'31.24"E
11		Hrabětice (South Moravia)	48°47'51.55"N, 16°23'36.33"E
12		Vranov (South Moravia)	49°18'35.37"N, 16°36'58.12"E
13		Pohořelice u Zlína (Zlínský)	49°10'32.59"N, 17°32'11.95"E
14		* Jelšava (Slovakia)	48°37'38.00"N, 20°14'28.81"E
15	**Blue spruce**	Praha 8	50°06'44.37"N, 14°28'31.24"E
16	*(Picea pungens)*	Radňoves (Vysočina)	49°23'19.02"N, 16°12'50.29"E
17		Hrabětice (South Moravia)	48°47'51.55"N, 16°23'36.33"E

Tab. 20 (continued): Analysed samples of needles – description

No.	Species	Sampling locality (region)	GPS
18	**Blue spruce**	Česká Třebová (Pardubický)	49°54'00.45"N, 16°25'49.51"E
19	*(Picea pungens)*	Česká Třebová (Pardubický)	49°54'09.31"N, 16°25'52.78"E
20		Vranov (South Moravia)	49°18'35.37"N, 16°36'58.12"E
21		Hlubočany (South Moravia)	49°13'51.77"N, 16°59'58.88"E
22		Prostějov (Olomoucký)	49°28'25.46"N, 17°09'44.94"E
23		Šelešovice (Zlínský)	49°15'17.19"N, 17°21'36.08"E

The following compounds were determined in the samples of needles:

- PCB congeners: 28, 52, 101, 118, 138, 153, and 180
- Organochlorine pesticides and other pollutants: HCB; α-, β- and γ-HCH; *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT

4.1.3. Tissue-specific distribution of organochlorine pollutants in birds of prey

This study was carried out in collaboration with Mendel University of Agriculture and Forestry Brno under a project "*Speciation of mercury in aquatic ecosystems and their transfer into food chains (2003-2005)*", and was aimed at the determination of levels of organochlorine contaminants in selected raptor species and their prey. Specimens of two aquatic species – the cormorant *(Phalacrocorax carbo)* and the grey heron *(Ardea cinerea)* – and of one terrestrial species – the buzzard *(Buteo buteo, Buteo lagopus)* – were collected in the region of Central Moravia. Since all the species are protected by law, their catching was done by workers of Moravian Ornithological Station in Přerov with a special permission. The investigated specimens came either from water reservoir near Záhlinice, which is a natural reserve established in 1995 and an important breeding place of water birds, or from the localities near the town of Nový Jičín (**Figure 31**). In order to assess the bioaccumulation and the biomagnification processes in the aquatic ecosystem, the concentrations of POPs in tissues of selected fish species collected in March 2003 were determined as well. The age of the fishes was between 2-4 years, their lengths ranged among 10.5-38.6 cm and the main species investigated were the common carp, the grass carp, the goldfish, the pike, the tech and the rudd. Pictures and descriptions in the following paragraphs are from [**221**,**224**].

The Great Cormorant *(Phalacrocorax carbo),* in Czech "Kormorán velký", is a large black bird, often over 100 cm in length with a wingspan of approx. 140 cm, which is very common and widespread across Afro-Eurasia and the Atlantic coast of North America. In the Czech Republic, main nesting areas include southern Bohemia and Moravia (e.g. Nové Mlýny Reservoirs). It roosts communally (up to hundreds of pairs in a colony depending upon the extent of surrounding feeding areas) and also flies and hunts its prey in flocks of varying sizes. The cormorant is a very greedy bird and its diet consists predominantly of fish as well as crustaceans, amphibians, molluscs and small birds. In the Czech Republic, cormorants are still protected by law as endangered species. However, their increasing populations have once again brought conflicts with fisheries for their flocks can do a lot of damage on marketable fish, especially over autumn months when northern populations migrate south across our country.

The Grey Heron *(Ardea cinerea)*, in Czech "Volavka popelavá", is a wading bird, native throughout temperate Europe and Asia and also parts of Africa. It is a large bird similar in appearance to the stork, standing about 100 cm tall, with a 175-195 cm wingspan and a weight of 1-2 kg. Herons breed in colonies or in sole pairs in trees close to lakes and feed in shallow water, catching fish or frogs with its long bill. Herons will also take small mammals and birds. The most numerous heron's populations in the Czech Republic can be found near Lednice (South Moravia) or Třeboň. Herons, just like cormorants, have ever been hunted as a varmint, but nowadays they are protected by law and their numbers rise again. The heron is partially a migratory species and the specimens from the Czech Republic usually winter in the Mediterranean or West Africa.

The Common Buzzard *(Buteo buteo)* and **the Rough-legged Buzzard** *(Buteo lagopus)*, in Czech "Káně lesní" and "Káně rousná", are both common species whose range covers most of Europe and extends into Asia. Buzzards are usually about 55 cm long with a 117-137 cm wingspan and female buzzards are often a bit bigger than males. Compared to the Common Buzzard, the Rough-legged Buzzard is longer-winged, more eagle-like in appearance, and it only winters in the Czech Republic. The buzzard breeds in woodland, but favours hunting over open land. It is very territorial and fights break out if one strays on another pair's territory. As a diurnal predator, the buzzard catches mainly small rodents as field mice (daily up to 10 mice) or hamsters, however, being suspected of hunting pheasants or fowls, buzzards are sometimes killed illegally as a varmint. Buzzards are used to live in pairs that mate for live. In the Czech Republic, the buzzard is protected by law as endangered species.

The following compounds were determined in samples:

- Indicator PCB congeners: 28, 52, 101, 118, 138, 153, 180
- Other important PCB congeners: 18, 44, 49, 70, 74, 87, 99, 105, 128, 151, 156, 170, 177, 183, 187, 194, 201
- Organochlorine pesticides and other pollutants: HCB; α-, β-, γ-, and δ-HCH; *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT; octachlorostyrene (OCS), Methoxychlor, Mirex, Aldrin, Dieldrin, Endrin, Isodrin, Heptachlor, cis-, trans- and oxy-Chlordane

Tab. 21: Detailed description of investigated specimens of raptor species

Date of death	Species (number)	Locality	Sex	Age	Analysed tissues
2003**	**Common Buzzard** (n=5)	Záhlinice Ponds	5 × ♀	<1	Muscle
2003**	**Rough-legged Buzzard** (n=1)	Záhlinice Ponds	♀	<1	Muscle
11/2004	**Great Cormorant** (n=6)	Hustopeče n/Bečvou	2 × ♂ 3 × ♀	±1**	Muscle, liver
11/2004	**Common Buzzard** (n=6)	Bartošovice	3 × ♂ 3 × ♀	1+	Muscle, liver
12/2004	**Common Buzzard** (n=2)	Bartošovice	2 × ♀	±1**	Muscle, liver
-/2004	**Grey Heron** (n=5)	Záhlinice Ponds	5 × ♀	±1**	Muscle, liver
09/2005	**Great Cormorant** (n=2)	Hustopeče n/Bečvou	1 × ♂ 1 × ♀	±1**	Muscle, liver
09/2005	**Grey Heron** (n=2)	Hustopeče n/Bečvou	1 × ♂ 1 × ♀	±1**	Muscle, liver
2007	**Great Cormorant** (n=8)	Záhlinice Ponds	8 × ♀	<1**	Muscles, liver, heart, kidney, brain, plumage, skin, intestinal contents

* n/a – not available; ** - estimated age/year

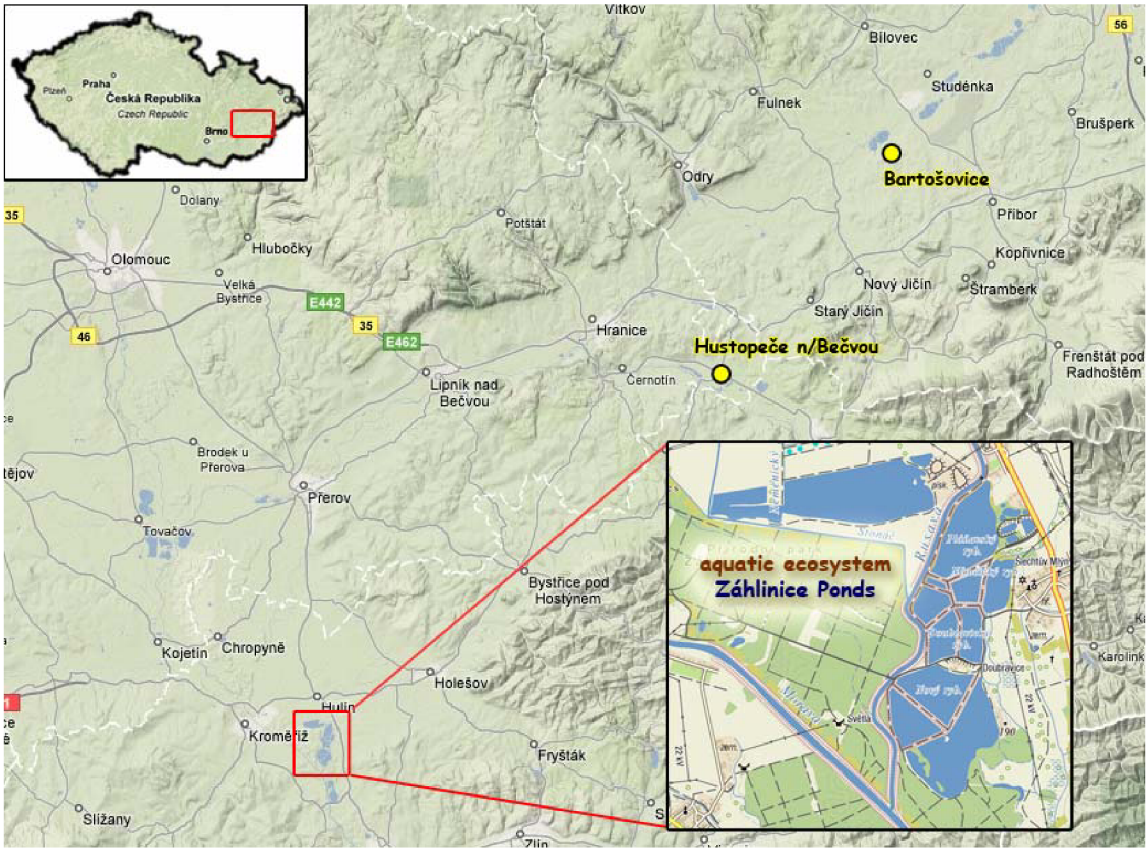


Fig. 31: Sampling localities of birds of prey specimens.

4.1.4. Comparison of microwave-assisted extraction and pressurised solvent extraction for the determination of organochlorines in various matrices

These experiments were aimed at two modern and relatively common extraction techniques – microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE) – and at the possibilities of their use in the determination of POPs in various samples. Testing matrices included two certified reference materials – BCR® No. 536 (Chlorobiphenyls in freshwater harbour sediment), No. 718 (PCBs in canned fresh herring) and real river sediment and agricultural soil. Extraction parameters as well as tested extraction solvents including *n*-hexane:acetone (1:1, v/v), *n*-hexane:acetone (7:3, v/v) and *n*-hexane:dichloromethane (1:1, v/v) were chosen from several recent papers dealing with this topic.

The following compounds were determined in the samples:

- PCB congeners: 28, 52, 101, 105, 118, 128, 138, 153, 156, 170, and 180
- Organochlorine pesticides and other pollutants: HCB; α-, β-, and γ-HCH; *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT

4.2. Chemicals & Materials

Organic solvents

- Acetone, dichloromethane, diethyl ether, isooctane, *n*-hexane, *n*-heptane, methanol, petroleum ether (Merck, Germany; SupraSolv® for GC)
- Acetone, dichloromethane, *n*-hexane, cyclohexane (Sigma-Aldrich, Switzerland; Pestanal® for GC residue analysis)
- *n*-Pentane (Penta Chrudim, Czech Republic; p.a. grade, redistilled before use)

Other chemicals & materials

- Anhydrous sodium sulphate powder, activated at 550 °C for 6 hours (Merck, Germany)
- Filter paper circles, ∅ 16.2 mm (Schleicher & Schuell, Germany)
- Florisil® (60-100 mesh), activated at 550 °C for 6 hours (Sigma-Aldrich, Switzerland)
- Silica gel 60 (70-230 mesh), purified with DCM and *n*-hexane, and activated at 200 °C for 3 hours (Merck, Germany)
- Silver nitrate ($AgNO_3$), sodium hydroxide (NaOH) and copper sulphate pentahydrate ($CuSO_4.5H_2O$) (Lachema, Czech Republic; p.a.)
- Sulphuric acid, 96% (Merck, Germany)
- Zinc powder (<63 μm particles) (BangCo Ltd., Czech Republic)

Technical gases

- Nitrogen 5.0, carbon dioxide 3.0 (Messer, Czech Republic)
- Hydrogen (>99.999%) from Dominick Hunter 20H Generator (Dominick Hunter, United Kingdom)
- Hydrogen 5.3, nitrogen 5.3 (Linde Gas, Czech Republic)

Standards of analytes

- PCB congener & OCP standard mix (**Pesticide-Mix 13**, Dr. Ehrenstorfer), 10 μl/ml in cyclohexane, containing PCB congeners No. 28, 52, 101, 138, 152 and 180; Aldrin, Chlordane (cis-, trans-, oxy-), DDT (4,4'; 2,4'), DDE (4,4'; 2,4'), DDD (4,4'; 2,4'), Dieldrin, Endosulfan (α-,β-), Endrin, HCH (α-,β-,γ-,δ-,ε-), Heptachlor, HCB, Isodrin, Methoxychlor, Mirex, and used for the preparation of standard calibration solutions: 0.0005 μg/ml, 0.001 μg/ml, 0.005 μg/ml, 0.01 μg/ml, 0.02 μg/ml, 0.05 μg/ml, 0.1 μg/ml and 0.5 μg/ml in cyclohexane.

- PCB Congeners Mixes (**Mix #2 – Mix Type Inducers, Mix #3 – PB Type Inducers, Mix #4 – Non Inducers Type**, AccuStandard), 10 μl/ml in *iso*-octane each, containing: **#2** (PCB No. 105, 118, 128, 138, 156, 170), **#3** (PCB No. 87, 99, 101, 153, 180, 183, 194), and **#4** (PCB No. 18, 44, 49, 52, 70, 74, 151, 177, 187, 201). For calibration purposes, mixed standard solutions of 0.0005 μg/ml, 0.001 μg/ml, 0.005 μg/ml, 0.01 μg/ml, 0.020 μg/ml, 0.1 μg/ml and 0.5 μg/ml in isooctane were prepared.

- **Pesticide Surrogate Spike Mix** (Sigma-Aldrich), 200 μg/ml each component in acetone, containing PCB 209 and 2,4,5,6-tetrachloro-*m*-xylene. For laboratory purpose, a stock solution **PSSM 10** (10 μg/ml in isooctane) was prepared.

- **Octachloronaphthalene** (Sigma-Aldrich) in solid state (ampule of 20 mg). For laboratory purpose, an internal standard solution **OCN1** (1 μg/ml in isooctane) was prepared.

Certified reference materials

- **BCR® 536** – Chlorobiphenyls in freshwater harbour sediment (Community Bureau of Reference – BCR®, Brussels, Belgium)
- **BCR® 718** – Chlorobiphenyls in Canned Fresh Herring (Community Bureau of Reference – BCR®, Brussels, Belgium)
- **Metranal™ 7** – PCBs, OCPs and PAHs in sewage sludge (Analytika® Ltd., Czech Republic)
- **Metranal ™ 2** – PCBs, OCPs and PAHs in river sediment (Analytika®, Ltd., Czech Republic)

4.3. Instruments & Laboratory equipment

Sample preparation

- Accelerated Solvent Extraction System ASE® 100 (Dionex, USA)
- Evaporation System TurboVap® II (Caliper Life Sciences, USA)
- Extraction System B-811 Standard (Büchi Labortechnik AG, Switzerland)
- Laboratory shaker LT-2 (SKLÁRNY KAVALIER, Co. Ltd., Czech Republic)
- Mechanical shaker OS 10 basic (IKA®, Germany)
- Pressurised microwave extraction system Multiwave 3000 with Rotor 16SOLV and solvent safety system (Anton-Paar, Austria)
- Pressurised Solvent Extraction System *one*PSE (Applied Separation, USA)

- Rotary evaporator Rotavapor® R-205 with heating bath B-490 and electronic vaccum controller V-800 (Büchi Labortechnik AG, Switzerland)
- Ultrasonic bath Sonorex® Super (Bandelin Electronic, Germany)
- Ultrasonic bath Teson 4 (Tesla, Czech Republic)

Gas chromatography – System I *(ICTEP, Faculty of Chemistry, BUT)*

Hewlett-Packard 6890N series II GC with:

- ✓ Automatic injector HP 7683, syringe 10 μl (Hamilton, USA)
- ✓ Programmable temperature vaporisation inlet (PTV) with electronic pneumatics control (EPC) and CO_2 cooling
- ✓ Two ^{63}Ni micro-electron capture detectors (μ-ECD), N_2 as make-up gas
- ✓ Two fused silica capillary columns with different polarities and operated in parallel:
  - **HT-8** (SGE, USA): 50 m × 0,22 mm i.d., 0,25 μm film thickness, 8% Phenyl (equiv.) Polycarborane-siloxane (front detector)
  - **DB-17ms** (Agilent J&W, USA): 60 m × 0,25 mm i.d., 0,25 μm film thickness, (50%-phenyl)-methylpolysiloxane (back detector)

Gas chromatography – System II *(Czech Geological Survey Brno)*

Hewlett-Packard 6890 Series GC with:

- ✓ Automatic injector HP 6890, syringe 5 μl
- ✓ Cool on-column injector
- ✓ ^{63}Ni micro-electron capture detector (μ-ECD), N_2 used as make-up gas
- ✓ One non-polar fused silica capillary column:
  - **HP-5ms** (Agilent J&W, USA): 60 m × 0.25 mm i.d., 0,25 μm film thickness, (5%-phenyl)-methylpolysiloxane

Software for data processing and presentation of the results

- Agilent ChemStation software: Rev. A.08.03, A.10.01 and B.03.01 (Agilent, USA)
- MS Office Excel 2003 and MS Office Word 2003 (Microsoft Corporation, USA)
- Grapher 7.2 (GoldenSoftware, USA), Corel 13 (Corel Corporation, USA)
- ACD ChemSketch 5.12 (Advanced Chemistry Development, Inc.)

4.4. Analytical methods

4.4.1. Determined compounds overview

Indicator PCB congeners

PCB 28	2,4,4'-Trichlorobiphenyl	CAS: 7012-37-5
PCB 52	2,2',5,5'-Tetrachlorobiphenyl	CAS: 35693-99-3
PCB 101	2,2',4,5,5'-Pentachlorobiphenyl	CAS: 37680-73-2
PCB 118	2,3',4,4',5-Pentachlorobiphenyl	CAS: 31508-00-6
PCB 138	2,2',3,4,4',5'-Hexachlorobiphenyl	CAS: 35065-28-2
PCB 153	2,2',4,4',5,5'-Hexachlorobiphenyl	CAS: 35065-27-1
PCB 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	CAS: 35065-29-3

Toxic and minority PCB congeners

PCB 18	2,2',5-Trichlorobiphenyl	CAS: 37680-65-2
PCB 44	2,2',3,5'-Tetrachlorobiphenyl	CAS: 41464-39-5
PCB 49	2,2',4,5'-Tetrachlorobiphenyl	CAS: 41464-40-8
PCB 70	2,3',4',5-Tetrachlorobiphenyl	CAS: 32598-11-1
PCB 74	2,4,4',5-Tetrachlorobiphenyl	CAS: 32690-93-0
PCB 87	2,2',3,4,5'-Pentachlorobiphenyl	CAS: 38380-02-8
PCB 99	2,2',4,4',5-Pentachlorobiphenyl	CAS: 38380-01-7
PCB 105	2,3,3',4,4'-Pentachlorobiphenyl	CAS: 32598-14-4
PCB 128	2,2',3,3',4,4'-Hexachlorobiphenyl	CAS: 38380-07-3
PCB 151	2,2',3,5,5',6-Hexachlorobiphenyl	CAS: 52663-63-5
PCB 156	2,3,3',4,4',5-Hexachlorobiphenyl	CAS: 38380-08-4
PCB 170	2,2',3,3',4,4',5-Heptachlorobiphenyl	CAS: 35065-60-6
PCB 177	2,2',3,3',4',5,6-Heptachlorobiphenyl	CAS: 52663-70-4
PCB 183	2,2',3,4,4',5',6-Heptachlorobiphenyl	CAS: 52663-69-1
PCB 187	2,2',3,4',5,5',6-Heptachlorobiphenyl	CAS: 52663-68-0
PCB 194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl	CAS: 35694-08-7
PCB 201	2,2',3,3',4,5,5',6'-Octachlorobiphenyl	CAS: 52663-75-0

Organochlorine pesticides & other compounds *(in alphabetical order)*

Aldrin	1,2,3,4,10,10-hexachloro-1,4,4α5,8,8α-hexahydro-exo-1,4-endo-5,8-dimethano-naphthalene	CAS: 309-00-2
cis-Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene	CAS: 5103-71-9
trans-Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene	CAS: 5103-74-2
oxy-Chlordane	1-exo-2-endo-4,5,6,7,8,8-octachloro-2,3-exo-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene	CAS: 27304-13-8
o,p'-DDD	1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane	CAS: 53-19-0
p,p'-DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane	CAS: 72-54-8
o,p'-DDE	1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene	CAS: 3424-82-6
p,p'-DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene	CAS: 72-55-9
o,p'-DDT	1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-ethane	CAS: 789-02-6
p,p'-DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane	CAS: 50-29-3
Dieldrin	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene	CAS: 60-57-1
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α -octahydro-endo,endo-1,4:5,8-dimethanonaphthalene	CAS: 72-20-8
α-HCH	1,2,3,4,5,6-hexachlorocyclohexane	CAS: 319-84-6
β-HCH	1,2,3,4,5,6-hexachlorocyclohexane	CAS: 319-85-7
γ-HCH	1,2,3,4,5,6-hexachlorocyclohexane	CAS: 58-89-9
δ-HCH	1,2,3,4,5,6-hexachlorocyclohexane	CAS: 319-86-8

Heptachlor	1,4,5,6,7,8,8α-hepta-chloro-3α,4,7,7α-tetrahydro-4,7-methanoindene	CAS: 76-44-8
HCB	Hexachlorobenzene	CAS: 118-74-1
Isodrin	1,2,3,4,10,10-Hexachloro-1,4,4α,5,8,8α-hexahydro-1,4:5,8-endo,endo-dimethanonaphthalene	CAS: 465-73-6
Methoxychlor	1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane	CAS: 72-43-5
Mirex	1,1α,2,2,3,3α,4,5,5,5α,5β,6-dodecachloro-octahydro-1,3,4-metheno-1H-cyclobuta-[*cd*]pentalene	CAS: 2385-85-5
OCS	Octachlorostyrene	CAS: 29082-74-4

4.4.2. Transfer and accumulation of selected POPs within aquatic ecosystems

Determined compounds

- PCB congeners No. 28, 52, 101, 118, 138, 153, 180
- α-, β-, γ-, δ-HCH; *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT; HCB, OCS

Analytical procedure

Selected organochlorine pollutants (PCBs and OCPs) were isolated from biological matrices, sediments and water samples by means of either pressurised solvent extraction (PSE) or Soxhlet extraction or liquid-liquid extraction (LLE). Crude extracts were then cleaned up on modified silica gel or Florisil® adsorption column. The final identification and quantification of target analytes was carried out using high resolution gas chromatography (HRGC) with electron capture detection (^{63}Ni μ-ECD), an eight-point power-fit calibration curve was used. The content of lipids in biological tissues was determined gravimetrically.

Extraction

- ✓ Water: the samples of water (1000 ml) were extracted in Erlenmeyer flasks with 30 ml of *n*-hexane using a shaker. The extraction was performed in three cycles, each for 20 min with new portion of solvent. The extracts were separated from water using a separator funnel and filtrated through a layer of anhydrous Na_2SO_4. Joined filtrates were then concentrated to about 2 ml using a rotary evaporator.

- ✓ Biotic tissues (fish muscle and viscera): between 3-30 g (depending on the amount of the sample or on the estimated lipid content) of homogenised tissue was mixed thoroughly with anhydrous Na_2SO_4 in order to make a powdery material. The sample was placed into an Erlenmeyer flask and extracted by 50 ml of petroleum ether (2 × 1 hr) using a shaker, the third extraction cycle was supported by sonication (15 min). Individual extracts were filtered through a layer of anhydrous Na_2SO_4. Joined filtrates were then concentrated to dryness using a rotary evaporator and the lipid content was determined gravimetrically. An aliquot (approx. 0.2 g) of extracted fat was then dissolved in 2 ml of *n*-hexane.

- ✓ Sediment, biotic samples (fish skin, phytoplankton): homogenised samples of sediment (~30 g), tissue (10-30 g) or phytoplankton (~4 g) were mixed with anhydrous sodium

sulphate powder, placed in a paper extraction thimble and extracted with petroleum ether for 6 hrs using accelerated Soxhlet extraction. The extracts were the evaporated to dryness using a rotary evaporator and dissolved in 2 ml of *n*-hexane. The determination of lipid content in fish skin was determined gravimetrically.

- ✓ Sediment (CGS): A sediment sample of 5 g was mixed with anhydrous Na_2SO_4 and loaded into a Dionex 10 ml stainless steel extraction cell. 25 µl of internal standard solution (PSSM 10) was added and the sample was subjected to the extraction by means of accelerated solvent extraction (ASE). Extraction was performed with DCM:methanol (93:7, v/v) at 80 °C and 2000 psi (i.e. ~140 bar), two static cycles (each 4 min) were carried out. After the extraction, the cell was flushed with solvent (60%) and purged with nitrogen (20 s). The extracts were then concentrated to about 5 ml under a gentle stream of nitrogen and adjusted to a final volume of 10 ml by DCM using a volumetric flask.

  The determination of total organic carbon (TOC) was performed in Laboratory of organic geochemistry of the Czech Geological Survey (CGS) and was based on a combustion of acidified samples at 1250 °C and infrared detection of CO_2.
  The dry weight of sediment samples was determined with a common gravimetrical method in the same laboratory.

Clean-up of the extracts

- ✓ Biotic tissues, phytoplankton, bottom sediments, water (ICTEP): 2 ml of crude extract was loaded on to a 45 cm × 20 mm glass column containing a glass wool plug, 2 cm of anhydrous Na_2SO_4, 5 cm of activated silica, 5 cm of activated Florisil and another 2 cm of Na_2SO_4. The column was pre-washed with 5 ml of *n*-hexane prior to use. The samples were eluted with 90 ml of *n*-hexane:diethyl ether (94:6, v/v). The eluates were then evaporated to 2 ml using a rotary evaporator. Samples that required additional purification (esp. biotic tissue extracts) were mixed with concentrated sulphuric acid (96%). The samples were then evaporated to about 0.5 ml, adjusted to a final volume of 1 ml with *n*-hexane and transferred to a GC vial.

- ✓ Sediment (CGS): For the clean-up procedure, 2 ml of the extract was taken to a vial and 2 ml of *n*-heptane together with 50 µl of internal standard OCN1 was added for the clean-up performance control. After the sample was concentrated to approx. 0.5 ml and adjusted to 2 ml with *n*-heptane, powder copper was added in order to remove sulphur (overnight). The clean-up was carried out on a 20 cm × 20 mm glass column which contained a glass wool plug, 1 cm of activated silica, 1 cm of silica impregnated with $AgNO_3$, 1 cm of silica impregnated with NaOH, another 1 cm of silica, then 3 cm of silica impregnated with 96% H_2SO_4 and finally 1 cm of silica. The sample was quantitatively transferred on the prepared column and the target analytes were eluted with 28 ml of pentane. The eluate was evaporated to 0.5 ml using a gentle stream of nitrogen, transferred to a GC vial and the volume was adjusted to 1 ml with *n*-heptane.

Gas chromatography analysis

The determination and quantification of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) was carried out by means of high resolution gas

chromatography (HRGC) with electron capture detection (μ-ECD). The two following GC setups were used:

✓ **GC System I** (water, sediment, fish tissues)

PTV injector programme	90 °C held for 0.1 min, then 750 °C/min to 350 °C, held for 5 min, then 10 °C/min to 220 °C
Detectors temperature (μ-ECD)	300 °C
Injected amount	2 μl
Oven temperature programme	Initial temperature 100 °C – increase to 195 °C at a rate of 20 °C/min – to 205 °C at 0.5 °C/min and held for 2 min – to 250 °C at 4.5 °C/min and held for 5 min – final increase to 300 °C at 7.5 °C/min and held for 10 min
Time of analysis	59 min
Carrier gas (flow, velocity)	Hydrogen (constant flow at 1.1 ml/min, 31 cm/s for the HT-8 column)
Make-up gas	Nitrogen (10 ml/min)

✓ **GC System II** (sediment)

Injector temperature	Cool on-column (Oven track mode)
Detector temperature (μ-ECD)	315 °C
Injected amount	1 μl
Oven temperature programme	Initial temperature 50 °C held for 2 min – increase to 150 °C at a rate of 50 °C/min – final increase to 300 °C at 5 °C/min and held for 11 min
Time of analysis	45 min
Carrier gas (flow, velocity)	Hydrogen (constant flow at 1.1 ml/min, 28 cm/s)
Make-up gas	Nitrogen (50 ml/min)

4.4.3. Needles as a suitable tool for the monitoring of POPs in the environment

Determined compounds

- PCB congeners No. 28, 52, 101, 118, 138, 153, 180
- α-, β-, γ-HCH; *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT; HCB

Analytical procedure

Selected organochlorine pollutants (PCBs and OCPs) were isolated from ground needles by means of cold solvent extraction. Crude extracts were then cleaned up on a mixed silica gel-Florisil® adsorption column. The final identification and quantification of target analytes was carried out using high resolution gas chromatography (HRGC) with electron capture detection (^{63}Ni μ-ECD), an eight-point power-fit calibration curve was used.

Extraction

Prior to extraction, needles were frozen deeply by liquid nitrogen and ground thoroughly in a mortar. An aliquot (10 g) of needles was then placed in an Erlenmeyer flask and extracted with 60 ml of *n*-hexane:acetone (3:2, v/v). The extraction was supported by shaking and three cycles (20 min each) were carried out. Individual extracts were filtered through a layer of

anhydrous Na_2SO_4. Joined filtrates were then concentrated to approx. 2 ml using a rotary evaporator.

Clean-up of the extracts

2 ml of crude extract was loaded on to a 45 cm × 20 mm glass column containing a glass wool plug, 2 cm of anhydrous Na_2SO_4, 5 cm of activated silica, 5 cm of activated Florisil and another 2 cm of Na_2SO_4. The column was pre-washed with 5 ml of *n*-hexane prior to use. The samples were eluted with 90 ml of *n*-hexane:diethyl ether (94:6, v/v). The eluates were then evaporated to about 0.5 ml, adjusted to a final volume of 1 ml with *n*-hexane and transferred to a GC vial.

Gas chromatography analysis

The determination and quantification of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) was carried out by means of high resolution gas chromatography (HRGC, System I) with electron capture detection (μ-ECD). Two different capillary columns were used for the separation of the target analytes (HT-8 and DB-17ms). An eight-point power-fit calibration curve (in the concentration range of 0.5-500 ng/ml for each compound) was used for the analytes quantification.

✓ **GC System I**

PTV injector programme	90 °C held for 0.1 min, then 750 °C/min to 350 °C, held for 5 min, then 10 °C/min to 220 °C
Detectors temperature (μ-ECD)	300 °C
Injected amount	2 μl
Oven temperature programme	Initial temperature 100 °C – increase to 195 °C at a rate of 20 °C/min – to 205 °C at 0.5 °C/min and held for 2 min – to 250 °C at 4.5 °C/min and held for 5 min – final increase to 300 °C at 7.5 °C/min and held for 10 min
Time of analysis	59 min
Carrier gas (flow, velocity)	Hydrogen (constant flow at 1.1 ml/min, 31 cm/s for the HT-8 column)
Make-up gas	Nitrogen (10 ml/min)

4.4.4. Tissue-specific distribution of organochlorine pollutants in birds of prey

Determined compounds

- PCB congeners No. 18, 28, 44, 49, 52, 70, 74, 87, 99, 101, 105, 118, 128, 138, 151, 153, 156, 170, 177, 180, 183, 187, 194, 201
- α-, β-, γ-, δ-HCH; *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT; HCB, OCS; Aldrin, Dieldrin, Endrin, Isodrin; Heptachlor, cis-, trans- and oxy-Chlordane, Methoxychlor, Mirex

Analytical procedure

Selected organochlorine pollutants (PCBs and OCPs) were isolated from tissues of raptors by means of either pressurised solvent extraction (PSE) or Soxhlet extraction. Crude extracts were then cleaned up on modified silica gel-Florisil® adsorption column. The final

identification and quantification of target analytes was carried out using high resolution gas chromatography (HRGC) with electron capture detection (^{63}Ni μ-ECD), an eight-point power-fit calibration curve was used. The content of lipids in biological tissues was determined gravimetrically.

Extraction

Two extraction procedures were performed for the isolation of the target compounds from raptors' tissues:

- ✓ Femoral and pectoral muscles, heart, kidney, liver: Homogenised samples were desiccated by mixing with anhydrous Na_2SO_4 and loaded into *one*PSE stainless steel extraction cell. Extractions were performed using petroleum ether with the following extraction conditions: 140 °C and 120 bar, three static cycles (3 min each), solvent flush 60%, purge time 1 min (N_2). The extracts were then concentrated to dryness using a rotary evaporator and the lipid content was determined gravimetrically. An aliquot (approx. 0.2 g) of extracted fat was then dissolved in 2 ml of *n*-hexane.
- ✓ Brain, intestinal contents, skin, plumage: Homogenised samples were mixed thoroughly with anhydrous Na_2SO_4, loaded into a extraction thimble and extracted with petroleum ether:*n*-hexane (1:1, v/v) for 6 hrs using accelerated Soxhlet extraction. After that the extracts were evaporated to dryness using a rotary evaporator and the lipid content was determined gravimetrically. An aliquot (approx. 0.2 g) of extracted fat was then dissolved in 2 ml of *n*-hexane.

Clean-up of the extracts

2 ml of crude extract was loaded on to a 45 cm × 20 mm glass column containing a glass wool plug, 2 cm of anhydrous Na_2SO_4, 5 cm of activated silica, 5 cm of activated Florisil and another 2 cm of Na_2SO_4. The column was pre-washed with 5 ml of *n*-hexane prior to use. The samples were eluted with 90 ml of *n*-hexane:diethyl ether (94:6, v/v). The eluates were then evaporated to 2 ml using a rotary evaporator and treated with concentrated sulphuric acid (96%) to remove the rest of co-extracted lipids. Upper layer of the sample was separated using a Pasteur pipette, evaporated to about 0.5 ml, adjusted to a final volume of 1 ml with *n*-hexane and transferred to a GC vial.

Gas chromatography analysis

The chromatographic conditions were consistent with those in Chapter 4.4.3.

4.4.5. Comparison of microwave-assisted extraction and pressurised solvent extraction for the determination of organochlorines in various matrices

Determined compounds

- PCB congeners No. 28, 52, 101, 105, 118, 128, 138, 153, 156, 170, 180
- α-, β-, γ-HCH; *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT; HCB

Extraction

The analysed certified reference materials and soil and sediment samples were extracted by means of microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE). Three different extraction mixtures were used.

✓ *Microwave-assisted extraction*

Prepared samples of soil, sediment or muscle tissue mixed with anhydrous Na_2SO_4 were loaded into PTFE extraction cylinders, and 25 ml n-hexane/acetone (1:1, v/v) or n-hexane/acetone (7:3, v/v) was added. Prior to the extraction, internal standard (PCB 203) was added to the samples. The extraction was performed at 115 °C for 20 min., followed by a cooling step so that the total extraction time neared 45 min. Microwave power was 1200 W (100%). During the extraction, the samples were mixed using a magnetic stirrer. The extracts were then filtered through a layer of anhydrous Na_2SO_4 in order to remove the extracted matrix.

✓ *Pressurised solvent extraction*

The samples were mixed with anhydrous Na_2SO_4 and loaded into 11 ml stainless steel cells which were partially filled with activated Florisil® in order to retain co-extracts. Internal standard (PCB 203) was added to all samples and extractions were performed with either *n*-hexane/acetone (1:1, v/v) or *n*-hexane/acetone (7:3, v/v) or *n*-hexane/DCM (1:1, v/v). The operating conditions were as follows: 100 °C with a 5 min heat-up time, 140 bar and two static extraction periods, each 5 min. After the extraction, the cell was flushed with solvent (60%) and purged with nitrogen (1 min). The extracts were collected into 30 ml glass vials with Teflon septa.

The extracts of both methods were evaporated to about 2 ml using a rotary evaporator, the rest of solvent was carefully removed by a gentle stream of nitrogen and the residue was dissolved in 5 ml of *n*-hexane.

Clean-up of the extracts

Extracts requiring further clean-up were evaporated to approx. 5 ml using a rotary evaporator, and loaded on to a glass column. The column contained a glass wool plug, 2 cm of activated Na_2SO_4, 20 cm of activated Florisil® and another 2 cm of Na_2SO_4. The column was conditioned with 10 ml *n*-hexane prior to use. The samples of sediment and soil were eluted with 90 ml *n*-hexane, extracts from fish tissue samples were eluted with *n*-hexan:diethyl ether (94:6, v/v). The eluates were then evaporated to 2 ml by means of a rotary evaporator and the rest of solvent was carefully removed by a gentle stream of nitrogen. The residues were dissolved in 0.5 ml n-hexane and transferred to a glass GC vial.

Gas chromatography analysis

The chromatographic conditions were consistent with those in Chapter 4.4.3.

5. RESULTS & DISCUSSION

5.1. Transfer and accumulation of selected POPs within aquatic ecosystems

The following chapters present the results from independent studies focused on the determination and assessment of accumulation of persistent organic pollutants in aquatic environments and food chains. The data were measured during 2007-2008 in the laboratories of Faculty of chemistry, BUT, and Czech Geological Survey. Water reservoirs and ponds from the Southern and Central Moravia regions were the localities of investigation.

5.1.1. Organochlorine pollutants in aquatic food chain of Brno Lake

Bioaccumulation of hydrophobic persistent contaminants in aquatic food webs is a well known phenomenon and this study was to show the process of magnification of POPs in the food chain of Brno Lake. Yet, owing to a limited number of the samples investigated (esp. fishes), the presented results must be viewed as just an example of possible bioaccumulation without any detailed statistical evaluation of links and correlations among the individual members of the food web.

Sketched in **Figure 32** are the assumed pathways of transport, deposition, redistribution and accumulation of PCBs to Brno Lake aquatic ecosystems. The scheme was based on the data from this study (fishes, zooplankton, water), present level of PCBs in the air (marked as **a**) was borrowed from [**13**] and the values of PCBs in sediment (**c**) and suspended particulate matter of the River Svratka (SPM, **b**) were obtained from Czech Geological Survey in Brno (with permission). Mean concentrations and proportions of PCBs, DDTs, HCHs and HCB in the investigated samples are presented in **Figure 33**.

In general, PCBs and DDTs were the most abundant organochlorines determined in all samples and their concentrations showed a relatively visible increase from water towards higher levels of the food chain. Whereas the amounts of PCBs and DDTs in water were 0.32 and 0.03 ppb (μg/L), respectively, in predatory fish species (zander) they increased to 3744 and 10510 ppb (μg/kg lipid weight), resp. There were no greater differences between the concentrations of PCBs and DDTs measured in bottom sediment (33 and 22 μg/kg dry weight, resp.) and those found in SPM from the River Svratka (22 and 18 μg/kg dw, resp.). A slightly higher level of PCBs (37 μg/kg ww) was found in the samples of zooplankton than in sediment. Higher ratio of PCBs/DDTs was calculated for water (11.1), sediment (1.5) and zooplankton (2.3), on the other hand, DDTs dominated in fishes, where the ratio was e.g. 0.4 in asp and 0.32 in perch. There was a strong positive correlation between the concentrations of PCBs and DDTs in all samples ($r = 0.954$).

As the levels of POPs changed through the food chain, the patterns of PCB congeners and DDT isomers and metabolites showed changes as well. **Figure 33** also brings relative abundances of individual PCBs and DDTs in the samples. As for PCBs, the most abundant congener in the water sample was PCB 28 accounting for about 70% of the total PCBs, while its contribution in asp fish was only 1.7% and higher chlorinated PCB 138, 153 and 180 were the predominant PCB congeners accounting for 77.2%. It is know that the PCB patterns shift from lower to higher chlorinated congeners when transferred to species positioned higher within the food chain [**122**]. Variances in the ratios of PCBs among fish species can be

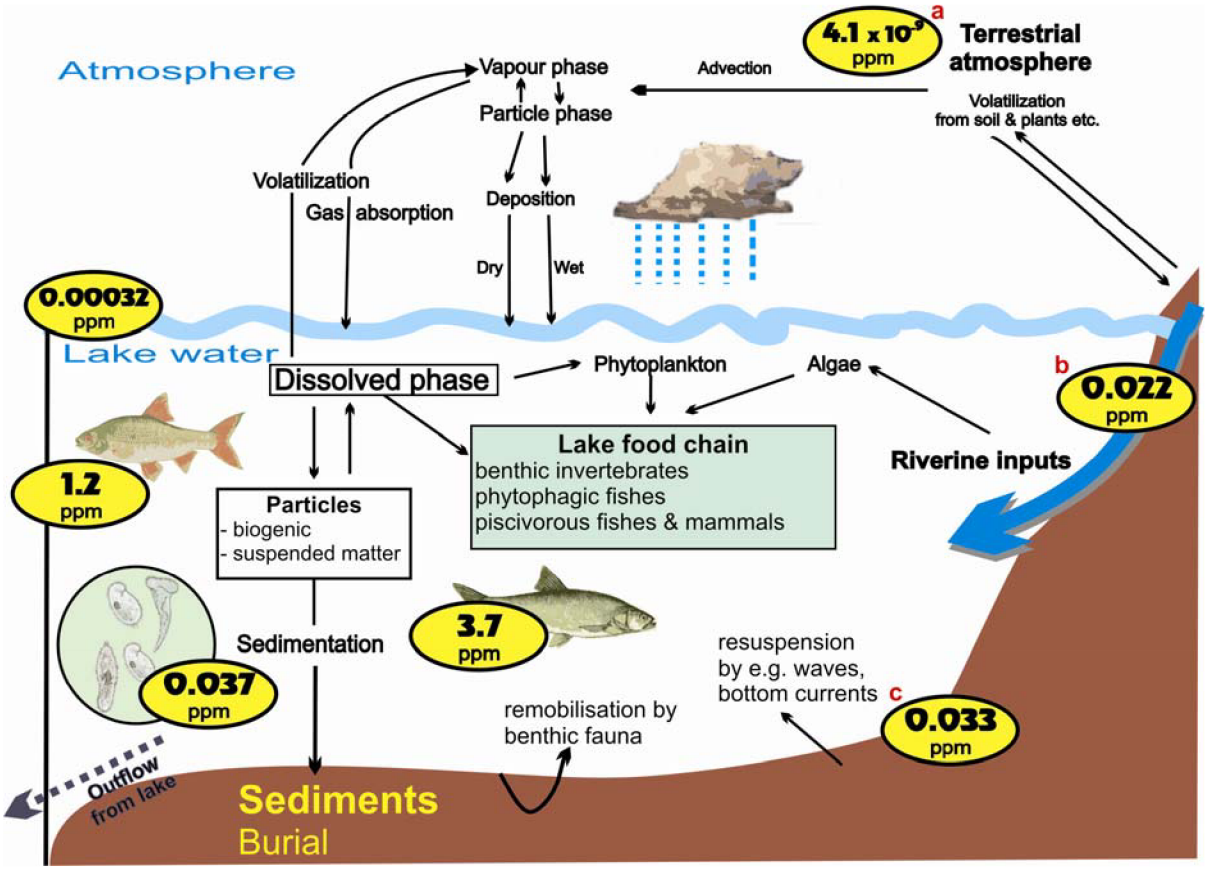


Fig. 32: Assumed pathways of transport, deposition, redistribution and accumulation of PCBs to Brno Lake aquatic ecosystems (basic scheme from [225]). ppm = mg/L (in water), mg/kg dw (sediment), mg/kg lw (fishes)

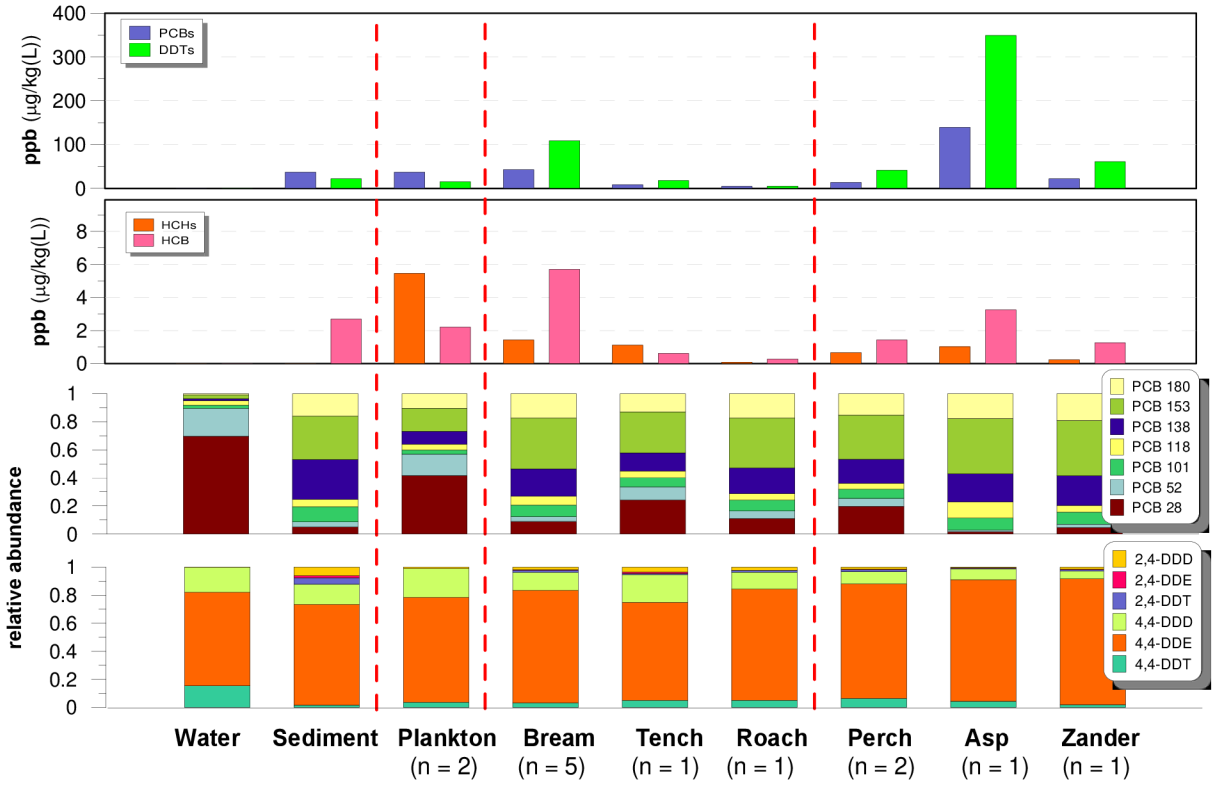


Fig. 33: Levels of organochlorine pollutants in abiotic and biotic samples from Brno Lake and the relative abundances of PCB congeners and DDT metabolites and isomers in the samples.

associated with their different feeding behaviour. Tench showing higher contribution of PCB 28 (24.2% of total PCBs) usually feeds on aquatic insect larvae and algae, whereas bream has higher dietary percentage of benthos (PCB 28 accounted for 8.8%). The difference between PCB patterns in sediment, where PCB 138, 153 and 180 accounted for 75.4%, and in plankton with 39.5% is also obvious. Plankton 'accumulate' POPs directly from water, where low chlorinated a more water-soluble PCB congeners dominate, while the adsorption of PCBs to fine particles of sediment is influenced by the content of total organic carbon (TOC) and high chlorinated and more hydrophobic (lipophilic) PCB congeners adsorb preferentially to particles and accumulate in adipose tissues. These findings can be well compared with similar data presented in **Chapter 5.3**.

p,p'-DDE was the most abundant compound of DDTs in all samples, followed by *p,p'*-DDD. As can be seen in the graph, the DDD/DDE ratio is somewhat higher in omnivorous fishes than in predators. In all samples, low proportions of *p,p'*-DDT were observed suggesting that there are no important current sources of this insecticide. A relatively high predominance of the aerobic *p,p'*-DDE metabolite in lake sediment is discussed in the following chapter.

The levels of HCHs and HCB determined in the samples were much lower than those of PCBs and DDTs (**Figure 33**), mainly in units of μg/kg, except water, where the levels of HCHs and HCB were 0.0048 and 0.0013 μg/L, respectively. Contrary to the increasing trends of PCBs and DDTs through the food chain, HCHs and HCB did not show any rise. As described further in **Chapter 5.3**, HCHs and HCB seem to differ from the biomagnification pathways of PCBs and DDTs and do not increase their concentrations in the food web. β-HCH accounted for the majority in the sediment samples, whereas γ-HCH was the only isomer detected in water. In fishes, α- and γ-HCH were the predominant isomers, accounting for on average 34% and 41.9%, respectively.

Fishes evaluated in this study are common species used for the monitoring of POPs in the aquatic ecosystems in the Czech Republic. Just for instance, mean levels of organochlorines in breams from Orlík Lake and the River Labe measured in 2006 were 9.8 and 94 μg.kg ww for PCBs and 22 and 173 μg.kg ww for DDTs, 63.4 μg.kg ww of PCBs in asp from Labe and 5 μg.kg ww in common carp from Orlík Lake [**262**]. Breams from Nové Mlýny reservoirs (2002) showed the PCB levels in range from 27 to 107 μg.kg ww and pike perch had the load with PCBs of 7-12 μg.kg ww [**249**]. The results from this study can be also compared to those presented in **Chapter 5.1.3** (POPs in chub from the River Svratka), **5.1.4** (Organochlorines in fish from recreational fishing) or **5.3** (Bioaccumulation of POPs in the Záhlinice Ponds) which show similar trends and levels of organochlorine pollutants.

In conclusion, despite limited set of samples, the results of this study can provide useful information on the current contamination of the food chain of Brno Lake and on the bioaccumulation and magnification of persistent organochlorine pollutants in sediment and aquatic biota.

5.1.2. Historical trends of POPs recorded in sediment cores from Brno Lake

River and lake sediments act as a sink for hydrophobic organic pollutants having high K_{OC} a strong affinity to particles. Therefore, sedimentary layers can provide good information on changes in contamination with POPs over decades. Contrary to surface sediments, analyses of POP concentrations in vertical sediment cores are carried out in much less extent, probably due to their technical demands. There are only few foreign articles on this issue compared to those focused on surface sediments, but in the Czech Republic no detailed survey on sediment cores concerning organochlorines has not been performed so far. This study was therefore aimed at the determination of historical changes in POPs recorded in sedimentary layers of Brno Lake and was carried out by Czech Geological Survey Brno in 2007-2008.

The concentrations of PCBs and other organochlorine pollutants determined in five sediment cores of various depths are shown in **Figure 34**. The graphs show the changes in concentrations of POPs depending on depth. The total organic carbon (TOC) content is provided as well. An overall bottom-to-top trend of PCBs in the deepest BP4 core is characterised by a slow increase of concentrations starting at a depth of about 200 cm and continuing as far as the interval of 70-77.5 cm where the maximum values of 76.7 and 77.6 μg/kg dw of ΣPCBs were determined. From this point up the levels of PCBs decline to the value of 39.4 μg/kg dw found in top 10 cm layer, representing few recent years. A significant leap in concentration of PCBs was observed at a depth of 160 cm, however, this correlated well with higher TOC content. Within the interval of 217.5-290 cm, the values of PCBs were very low and just above LOQ. Based on the determined TOC changes, the interval represents fluvial environment with organic lean and sandy sediments. The observed increase in the TOC (200-216 cm) suggests (among others) some important change in sedimentation and we assumed it to represent the period of time of the first filling the reservoir (i.e. the late 1930s). From this depth up the TOC content varies from 2.1% to 7.2%. Presented in **Figure 41** is a comparison between the vertical levels of PCBs in the BP4 core and the trends in production and utilisation of PCB mixtures in the former Czechoslovakia. When our results are compared to the historical records on the PCB use, we can observe some phenomena. First, the manufacture together with their utilisation in the former Czechoslovakia started in the early 1960s and terminated was in 1984. Thus, the occurrence of PCBs in lower sedimentary layers may suggest that PCB products (of foreign origin) had been used in the Czechoslovakia before the date their domestic production in Chemko Strážské (Slovakia) began. The beginning of the industrial production and use in the world started in the 1930s. Evenset et al. [**252**] also described the presence of PCBs in a dated core from one Norwegian Arctic lake. They explain the occurrence of PCBs in 'pre-industrial' segments of the core as the results of (I) molecular diffusion, (II) sediment coring artefacts and (III) bioturbation. Since the BP4 core was taken at the beginning of Brno Lake where the changes in water level strongly affect the sedimentation process, and considering rich aquatic biota inhabiting this area, we assume drilling core and biota as the most likely causes. Unfortunately, the ^{137}Cs dating of the core exactly determining the age of the sediment layers was still on order by the time of submitting this thesis, thus, no exact conclusions can be drawn.

As for the other cores, different trends in PCBs were observed. The VB07 core (**Figure 34**), taken from the River Svratka just before its issuing in the lake, showed a strong relationship between the TOC content and PCB levels. Moreover, the TOC content was on average higher in this core and the concentrations of PCBs were thus higher than those in the

BP4 core. More dynamic sedimentation history of the VB07 core and less detailed division of the core caused that PCB levels in the profile did not exactly correspond with historical trends in PCB use, as recorded better in the BP4 core. The BP1-BP3 cores were sampled by means of a corer and only the BP1 core, taken just off the BP4, showed certain trend in PCBs. Since two cores were taken from the sampling sites for comparison, the (A) and (B) samples of each core are coloured in black and red, resp. Since the sedimentation rate at the sampling sites of the other BP2 and BP3 cores is significantly affected by boat traffic and performing water sports in this area, the profiles do not represent any general trends. However, only limited number of sub-samples was analysed.

The relative abundances of PCB congeners in the BP4 and VB07 cores are presented in **Figures 35**, **37** and **38**. In general, three predominant congeners in all cores were high chlorinated PCB 138, 153, and 180. The ratio of PCB congeners 28/153 in the BP4 core ranged from 0.05 to 1.6 with a mean of 0.27 and the congener pattern was relatively consistent throughout the whole length of the core. However, an obvious change in PCB patterns occurred at a depth of about 95 cm in the BP4 core. This individual and significant increase of the PCB 28/153 ratio (1.6) may suggest a different emission source of PCB, e.g. a technical mixture containing low chlorinated PCB congeners leaked to the lake.

The levels and historical changes of DDTs were relatively similar to those of PCBs in the BP4 core, but there was a sharper increase in total DDTs from the depth of 200 cm than that of PCBs (**Figure 34**). The maximum values of ΣDDTs were 72.4 and 68.7 μg/kg dw determined at depths of 128 and 70 cm, respectively. The concentrations of DDTs determined in the uppermost and most recent layers of both cores were about 20 μg/kg dw. As can be seen in the graph, in the BP4 core, the trends of DDTs are not as continuous as those of PCBs, but there are two distinct decreases at the depths of 108 and 93 cm. Since no significant variation of the TOC content was observed, such declines may reflect an important change in the sedimentology. For instance, the lower layer (approx. 100-120 cm) was composed of more sandy matter than the surrounding layers comprising organic rich sediments. This observation could suggest PCBs and DDTs have different emission sources and the historical event (probably flood) causing the change in sediment composition affected the concentration of DDTs but not of PCBs. In the VB07 core, the increase-maximum-decline trend is not as clear as in the other core, probably due to the same reasons described above. Shown in **Figure 36** and **39** are the relative abundances of individual DDTs in two deepest cores. The most interesting thing in the VB07 core was a substantial jump in *p,p'*-DDT the amount of which in at such depth (about 200 cm) seemed not probable. Although the chromatographic analysis did not show any anomaly, however, analytical error seems to be the most likely explanation.

Concentrations and proportions of DDT isomers were found to be in order DDE > DDD > DDT in almost all samples. Particularly, the concentration of *p,p'*-DDE, a higly stable aerobic breakdown metabolite of *p,p'*-DDT, dominated in all segments from both cores and accounted for 49.9-81.7% of ΣDDTs. This is somewhat unusual observation in lake sediments and it may indicate input of highly weathered DDT, as published by Evenset et al. [**252**]. The mean ratio of *p,p'*-DDT/*p,p'*-DDE, used to estimate the "age" of DDT inputs, was 0.13 in this study. A ratio of 0.33 or less is considered as aged mixture [**253**].

HCB levels in the BP4 core did not show any clear trends with depth. An increase in HCB concentrations was observed in the interval from 155 to 115 cm, from this depth upwards the levels of HCB did not vary a lot and showed constant values, probably due to continuous emissions. There was a decline in HCB at the depth of 108 cm, similar to DDTs.

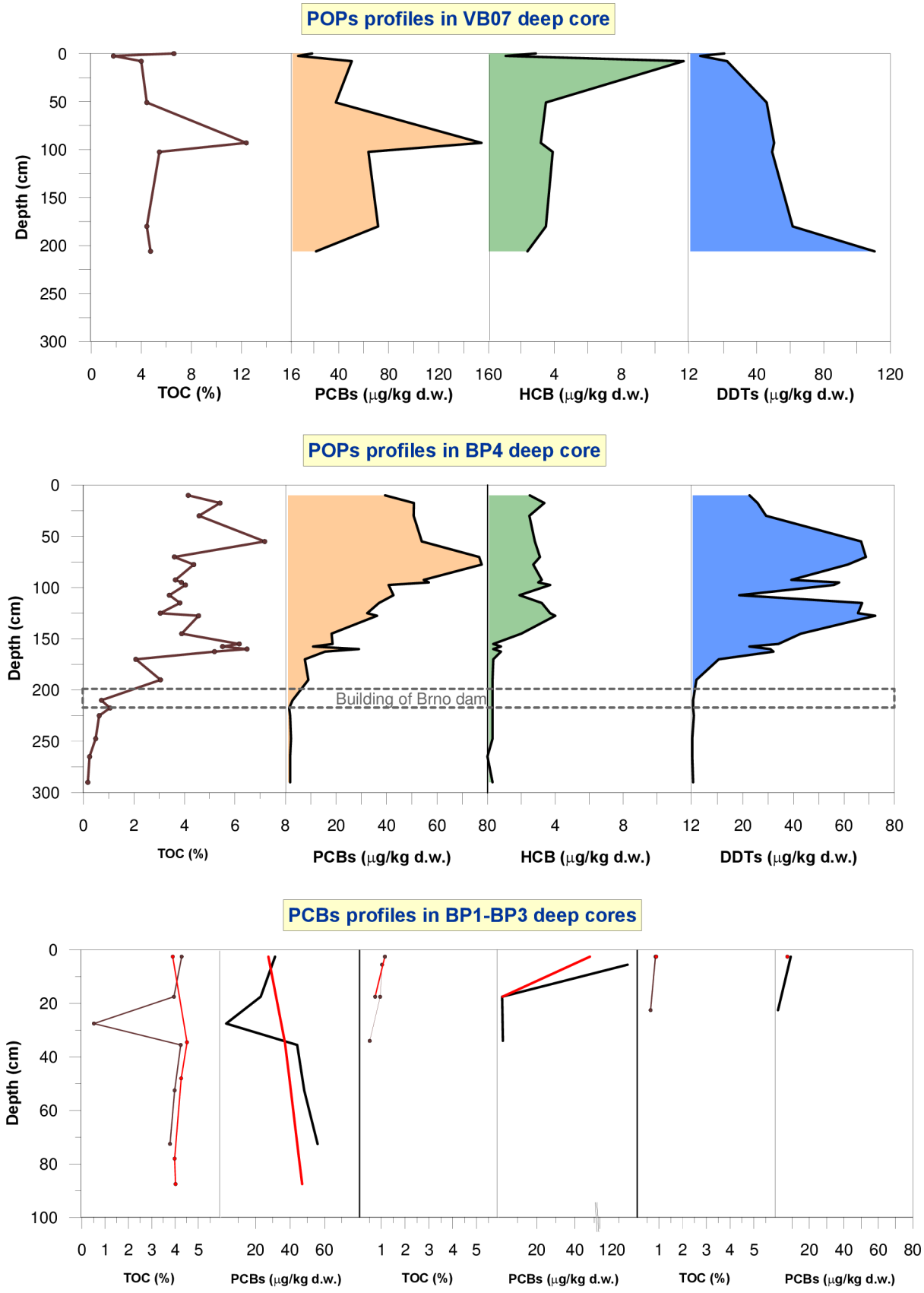


Fig. 34: Levels of POPs in deep core profiles of sediments from Brno Lake.

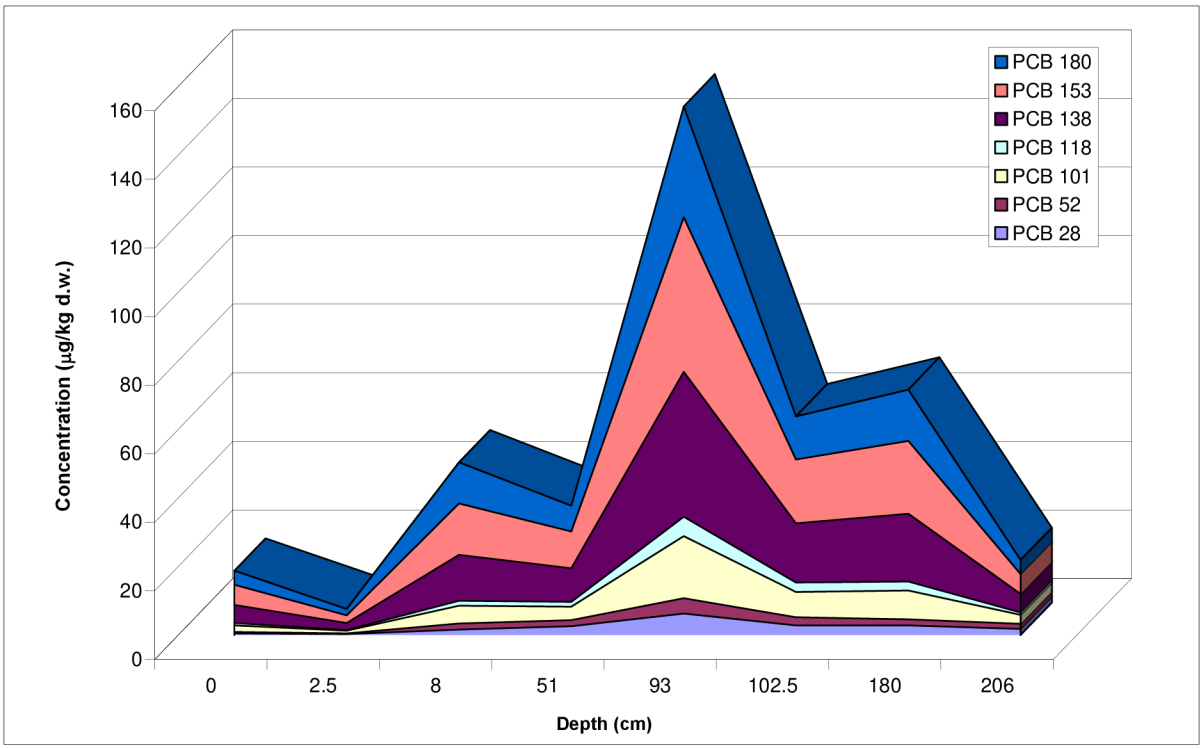


Fig. 35: Vertical distribution and ratios of PCB congeners in the VB07 core (Brno Lake).

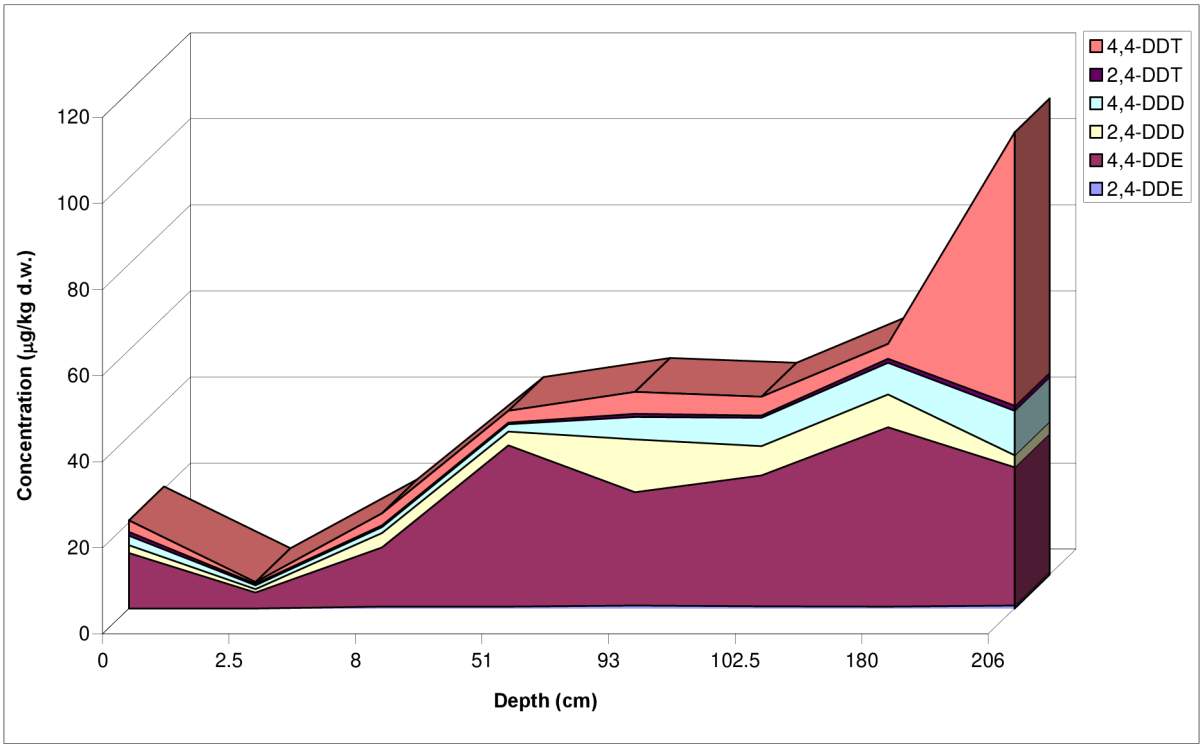


Fig. 36: Vertical distribution and ratios of DDTs in the VB07 core (Brno Lake).

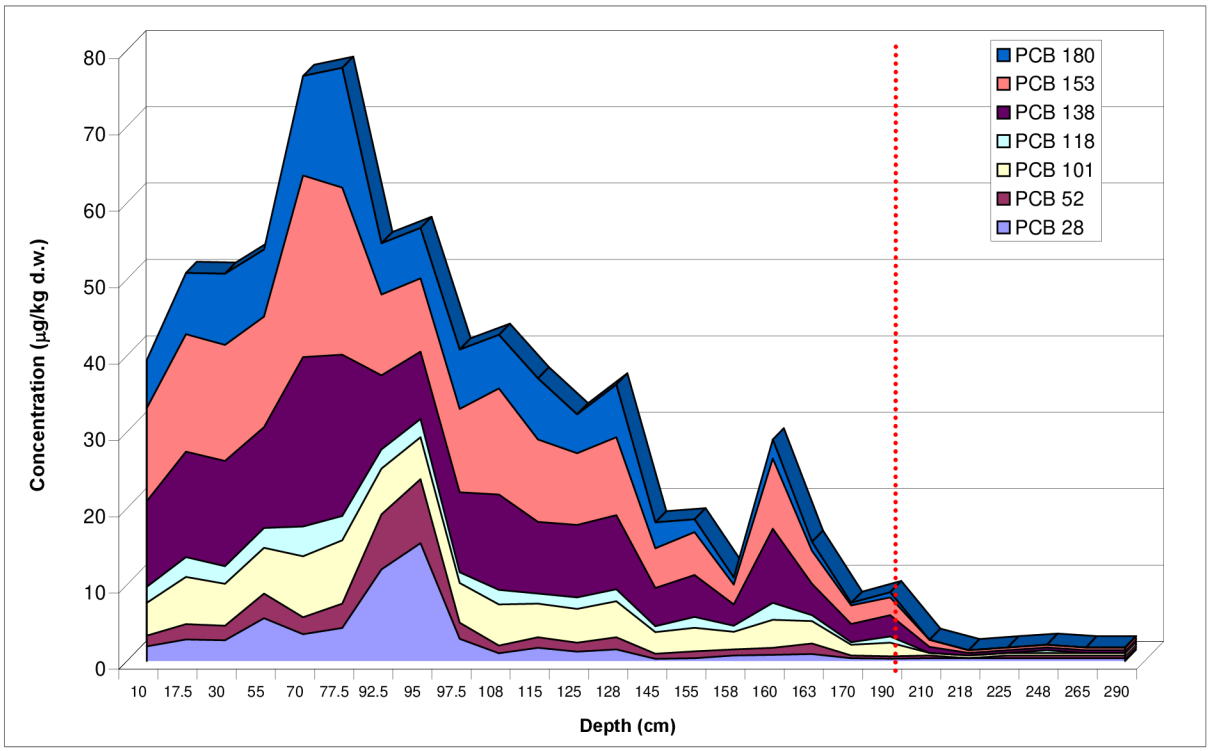


Fig. 37: Vertical distribution and ratios of PCB congeners in the BP4 core (Brno Lake).

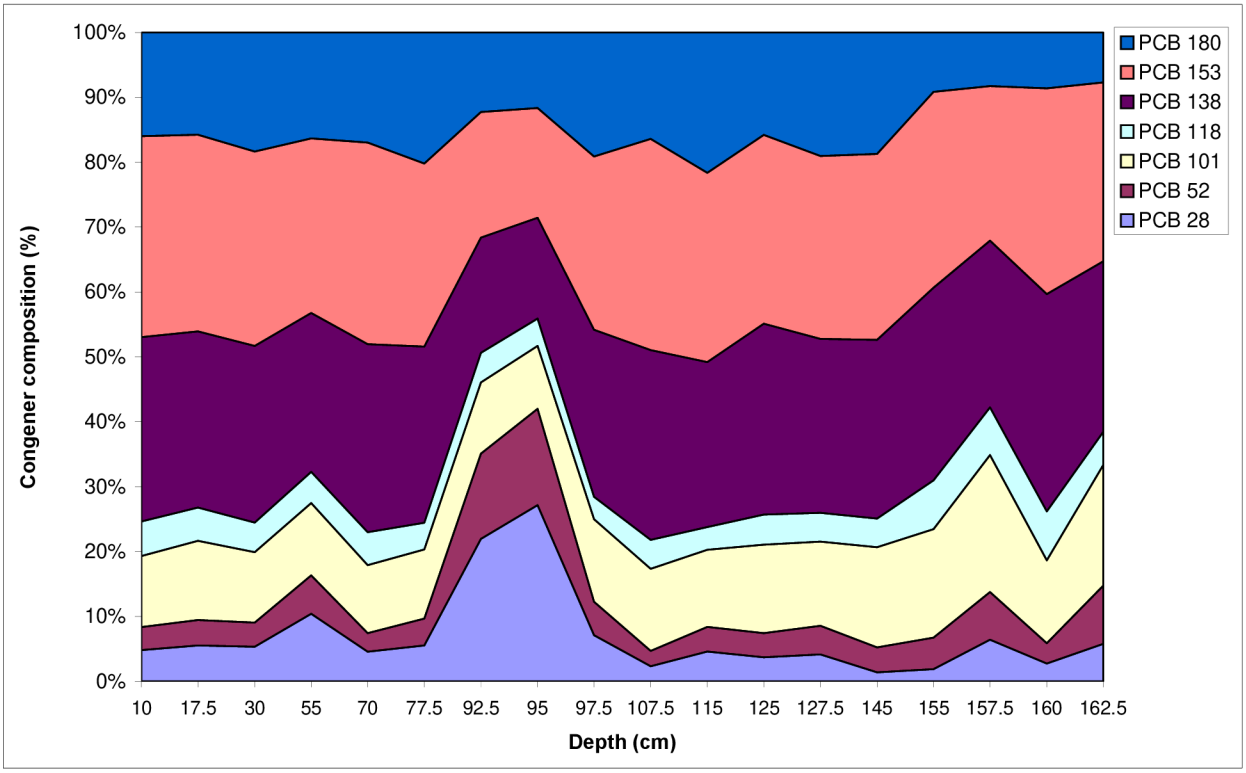


Fig. 38: Relative abundance of PCB congeners in the BP4 core (Brno Lake).

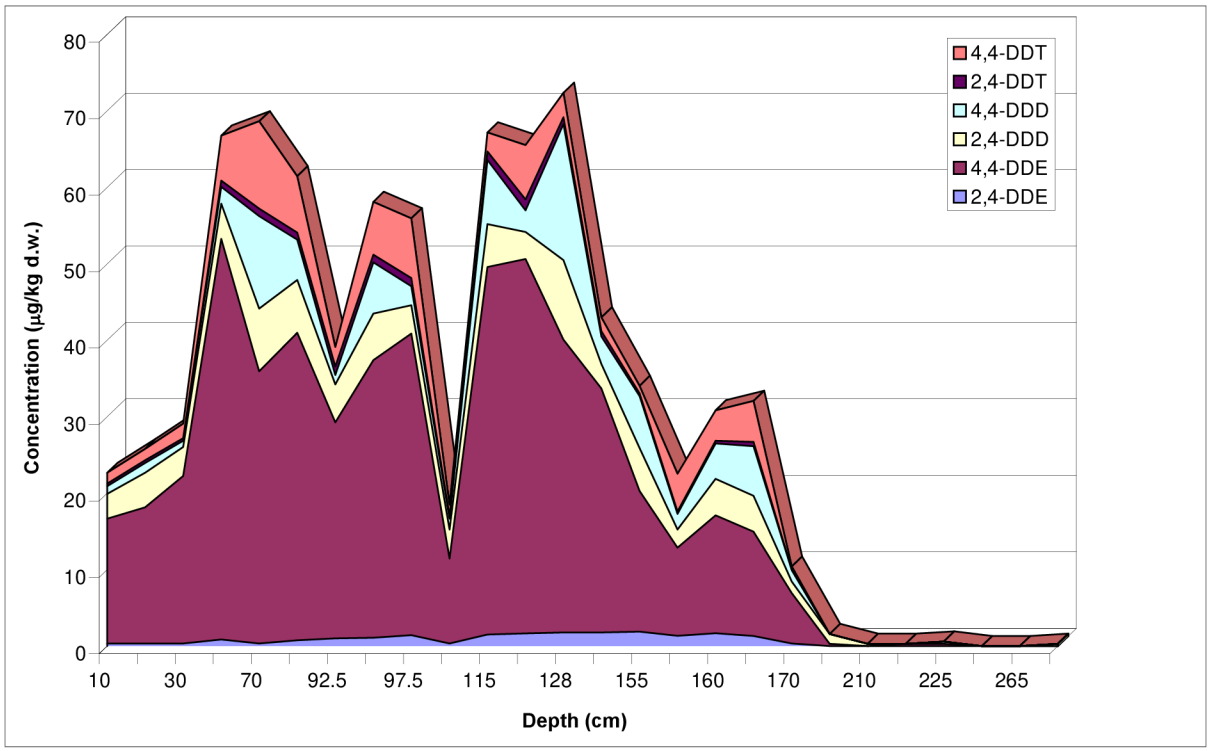


Fig. 39: Vertical distribution and ratios of DDTs in the BP4 core (Brno Lake).

Total HCHs (Σα-, β- and γ-HCH) in core slices were in the range from not detected to <0.9 µg/kg dw. The most detected isomers were the β- and α-HCH, however, the levels did not exceeded the LOQ values. Except one sample, the γ-HCH was not detected. The predominance of β̃-HCH in sediments can be caused by the α- and γ-HCH transformation [**254**]. Similarly, the β–HCH is by far the more persistent and less soluble in water than the other isomers. However, the levels of HCHs seemed not to be of greater importance in the studied sediments.

Simple regression analysis of the ln-transformed concentrations of PCBs, DDTs, HCB and total organic carbon (TOC) in individual sediment layers are shown in **Figure 40**. The correlation was relatively good for PCBs and DDTs, but poor for HCB. In fact, this is often used for the prediction of the levels of hydrophobic pollutants in soils and surface sediments depending on the TOC content [**258**]. However, to calculate such correlation, the compounds evaluated should be of the same emission history (surface sediments of identical age), not like in this study. Then, the correlation does not show a direct relationship between the concentrations of POPs and TOC, but it is rather such an approximation.

Determination of organochlorine pollutants in sediment cores from different localities of the world has been published in several papers. Frignani et al. [**259**] studied historical changes of organochlorine pollutants in sediment cores from the Venice Lagoon (Italy) and reported an increase of PCBs and HCB from the 1940s and 1900s, resp., with the maximums of 203 and 3.6 µg/kg dw in 1970 and 1980, resp. and then a decline in levels up to now. They also found PCBs in the sediment layers dated as before 1930s the production of PCBs started, which may be due some bioturbation and physical mixing, or due to e.g. waste incineration. Another similar recent survey by Zennegg et al. [**260**] determined PCB deposition at Greifensee, Switzerland. The PCB levels started to increase after 1940 to the maximum value

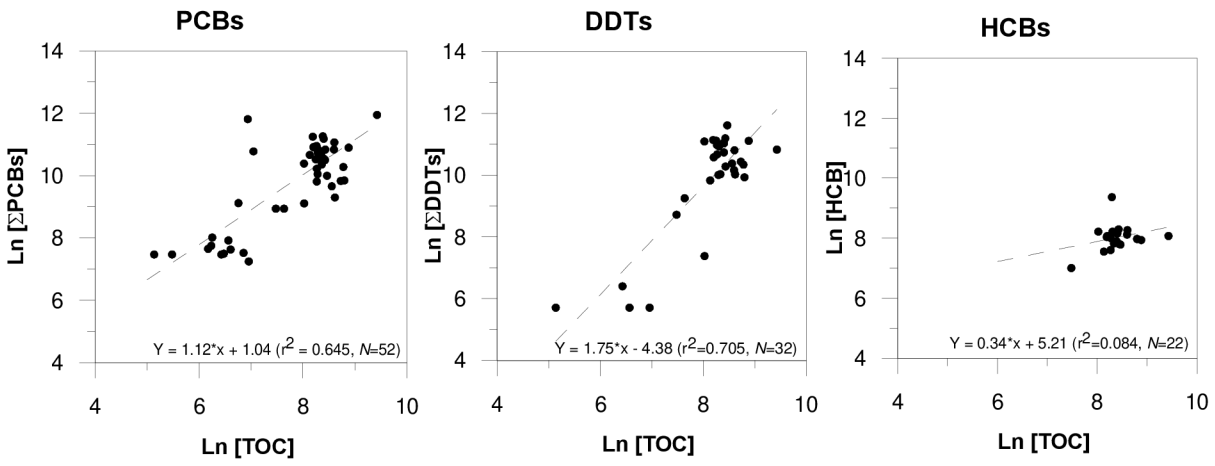


Fig. 40: Relationship between the ln-transformed concentrations (µg/kg dw) of ΣPCBs, ΣDDTs, HCB and total organic carbon (TOC) in sediment samples from deep cores from Brno Lake 2007-8.

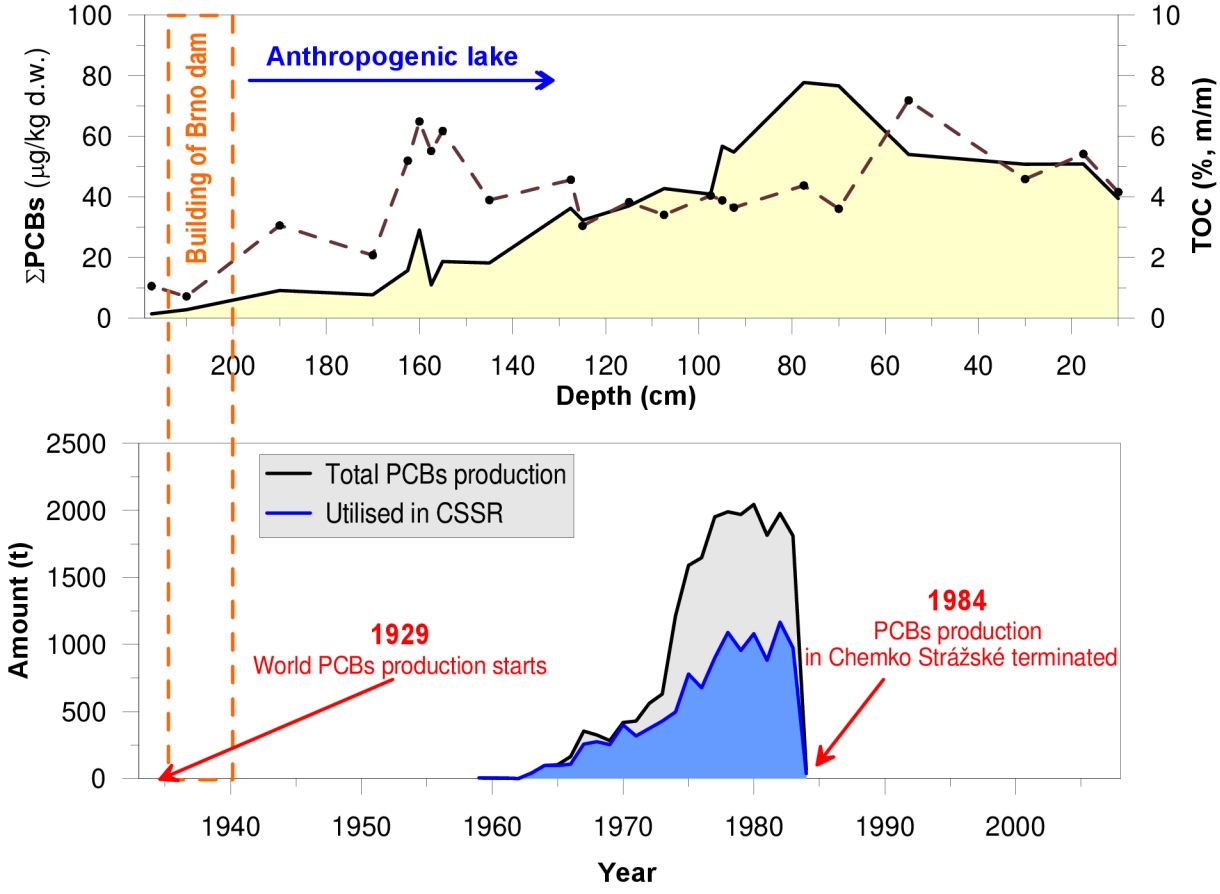


Fig. 41: Comparison between the vertical levels of PCBs in the BP4 core and the trends in production and utilisation of PCB mixtures in the former Czechoslovakia (historical data from [**13**]).

of 130 µg/kg dw in the early 1960s, the most recent sediment slice (1999) contained about 7 µg/kg dw of PCBs. And finally, Hong et al. [**261**] showed similar trends in PCBs and DDTs in sediment cores from Masan Bay, Korea. The peaks of both pollutants were dated as back as the 1980s and then decreasing trend was observed. This can be regarded as a general global trend reflecting the international efforts to limit the PCB and DDT use during past decades.

5.1.3. Organochlorine pollutants in chub *(Leuciscus cephalus)* from the Svratka River

This survey was focused on the determination of the levels of persistent organic pollutants in the chub which is a common species used as freshwater bioaccumulation marker. Detailed information on the specimens caught and analytical procedures are given in **Chapter 4.1.1.** Briefly, fish were sampled from the River Svratka at two localities (Modřice and Rajhradice), both of them situated downstream from the wastewater treatment plant of the Brno city. The sampling localities were divided mutually by a weird, so that the fish were not supposed to migrate between them. The main aim of this study was (I) to assess the current load of selected freshwater fish species with POPs in the River Svratka, (II) to compare differences in levels of POPs in fish from distant and not connected localities within the same watercourse and (III) to assess potential variations due to different sampling periods.

Shown in **Figure 42** are the concentrations of ΣPCBs in fish from two sampling localities and two sampling periods. Mean levels of PCBs in muscle of chubs from Modřice were 41.9 and 30.8 μg/kg ww for the spring and autumn sampling, respectively, and at Rajhradice, the means were determined as 38.8 and 38.6 μg/kg ww, resp. There were no statically significant differences in levels of PCBs between both sampling localities ($p < 0.05$) and between the sampling periods ($p < 0.05$) within each locality. Observed differences and varying ranges of values in individual sets of samples might be caused by different weights and metabolism of fish rather than by 'outer' factors including the assumed contribution of the wastewater treatment plant in Modřice (WWTP). An interesting value for a complex evaluation is the weighed mean, taking the different body weights of fish from each set into consideration. Then, the weighed means for the Modřice locality were calculated as 42.5 and 30.7 μg/kg ww (in spring and autumn, resp.) and for Rajhradice as 40.6 and 38.9 μg/kg ww (in spring and autumn, resp.). The former sampling area showed a decline in the value of weighed mean, however, the change was generally not of great significance and could be caused e.g. by abundance of non-contaminated food between two sampling periods. The decrease in weighed mean values is more marked at the locality of Modřice. Levels of PCBs determined in other two tissues of chub are presented in **Fig. 46**. The highest concentrations of PCBs ranging from 115.1 to 681.7 μg/kg ww were found in skin of all samples, followed by viscera with a range from 37.9 μg/kg ww to 559.7 μg/kg ww. The lipid content determining the levels of PCBs in the tissues was on average 13.2% in skin, 12.3% in viscera and 2.2% in muscle. Contrary to the relatively invariable lipid content in muscle and skin in all samples (on average 1.62%-2.96% in muscle and 9.93%-15.9% in skin), significantly higher lipid content in viscera ($p < 0.01$) was observed in specimens from the autumn sampling (12.42%-17.78%) than in those from the spring season (9.35%-9.58%) which was true for both sampling sites. Since the levels of PCBs in lipids from different tissues did not vary significantly ($p < 0.01$), the concentrations of PCBs in viscera of the 'autumn' specimens were similar to those in skin. This was also true for the other contaminants monitored. Higher lipid contents in viscera fish caught in the autumn might be explained by feeding activity over summer, whereas during hibernation in winter the fat reserves are metabolised to energy.

Predominating PCB congeners in all tissues were the higher chlorinated CB 138, 153, and 180. These three PCBs accounted for 58% and 64% of total ΣPCBs in the samples from spring and autumn, respectively. Relatively high abundance also showed PCB 28 with 20% and 16% in the spring and autumn fish, resp. Closer look at the distribution of individual PCBs shown in **Fig. 42** would discover that even within the same set of fish exist differences

in patterns of PCBs and variances in their relative abundances. Results from **Chapter 5.3** can serve as a comparison.

Concentrations of DDTs (**Fig. 43**) similar to those of PCBs were determined in the muscle tissue of chub specimens as well. Means of ΣDDTs in chub from Modřice were 34.8 (ranging from 8.4 to 78.1) and 28.6 (with range of 13.3-41.1) μg/kg ww for the spring and autumn sampling, respectively, and at Rajhradice, they were 38.1 (5.2-93.8) and 27.8 (2.9-47.5) μg/kg ww, resp. There were indeed strong correlations (r = 0.781-0.985) between DDTs and PCBs in all set of samples except the locality Mořice (autumn sampling), where the correlation was somewhat weaker (r = 0.468). On the whole, the values did not significantly differ between the sampling localities ($p < 0.01$) or between two sampling periods ($p < 0.01$), just like PCBs. The weighed means for the Modřice locality were 37.9 and 29.4 μg/kg ww (in spring and autumn, resp.) and in Rajhradice they were 40.0 and 28.8 μg/kg ww (in spring and autumn, resp.). The trend in decreasing values was similar to that described for PCBs. And again, due to higher lipid content in viscera of the specimens from the autumn sampling, the concentrations of PCBs in viscera were similar to those in skin (**Fig. 46**).

Among DDTs with chlorines located in the *p,p'*- positions, the metabolite DDE highly predominated in all tissues, followed by *p,p'*-DDD and *p,p'*-DDT the contributions of which were quite comparable. The *p,p'*-DDE accounted for on average 65.5-69.5% in the four sets of fish, the *p,p'*-DDD and *p,p'*-DDT isomers accounted for 14.6-17.8% and 9.4-11.2%, respectively. The ratio of DDT/DDE ranged from 0.04 to 0.25 (mean of 0.15) which suggests this load is relatively old and there are no longer significant sources of DDT. Just for comparison, Suchan et al. [**263**] monitored the levels of organochlorines in selected fish species from the Labe, Vltava and Tichá Orlice rivers in the years of 2001-2003 and reported the average ratio of 0.1 for DDT/DDE. Thus, the fish in our study do not show any divergence to a general trend in the Czech Republic. Further comparisons are in **Chapters 5.1.4** or **5.3**. The *o,p'*- isomers of DDTs made up a minor contribution to the total sum of DDTs.

Contrary to PCBs and DDTs, the concentrations of HCHs were very low with predominating β- and γ- isomers (**Figure 44**). The average values of ΣHCHs in muscle tissue of chub were all in units or rather tenths of μg/kg ww, except for the autumn samples from Rajhradice, where significantly higher values ($p < 0.01$) ranging from 0.2 to 5.3 (mean of 2.6) μg/kg ww were measured. The weighed means for the Modřice locality were 1.02 and 0.7 μg/kg ww (in spring and autumn, resp.) and 0.87 and 2.6 μg/kg ww (in spring and autumn, resp.) in Rajhradice. As can be seen in **Fig. 44**, only the γ-HCH (known as an insecticide *lindan* and used formerly in agriculture) showed a rise at this locality which could suggest some fresh source of this contaminant. But on the whole, this increase seems not to be of greater importance. The γ-HCH was the most abundant isomer through the whole set of samples accounting for on average 56.3% of the total HCHs except for the autumnal set of samples from Rajhradice, where it accounted for the above discussed 80.5%. The trend in proportions of α- and β-HCH isomers was not as clear as that of γ-HCH. Due to low levels of HCHs, just above the LOQ in many cases, no correlations to the other compounds measured were calculated.

HCB and OCS were the last two organochlorine pollutants determined in the fish. Similarly to the other pollutants, neither HCB nor OCS showed any differences of statistical importance ($p < 0.01$) between the two sampling localities and sampling periods. HCB was detected in the samples in much higher concentrations than OCS, ranging mostly just above LOQ. However, the HCB levels were in units of μg/kg ww, compared to PCBs and DDTs.

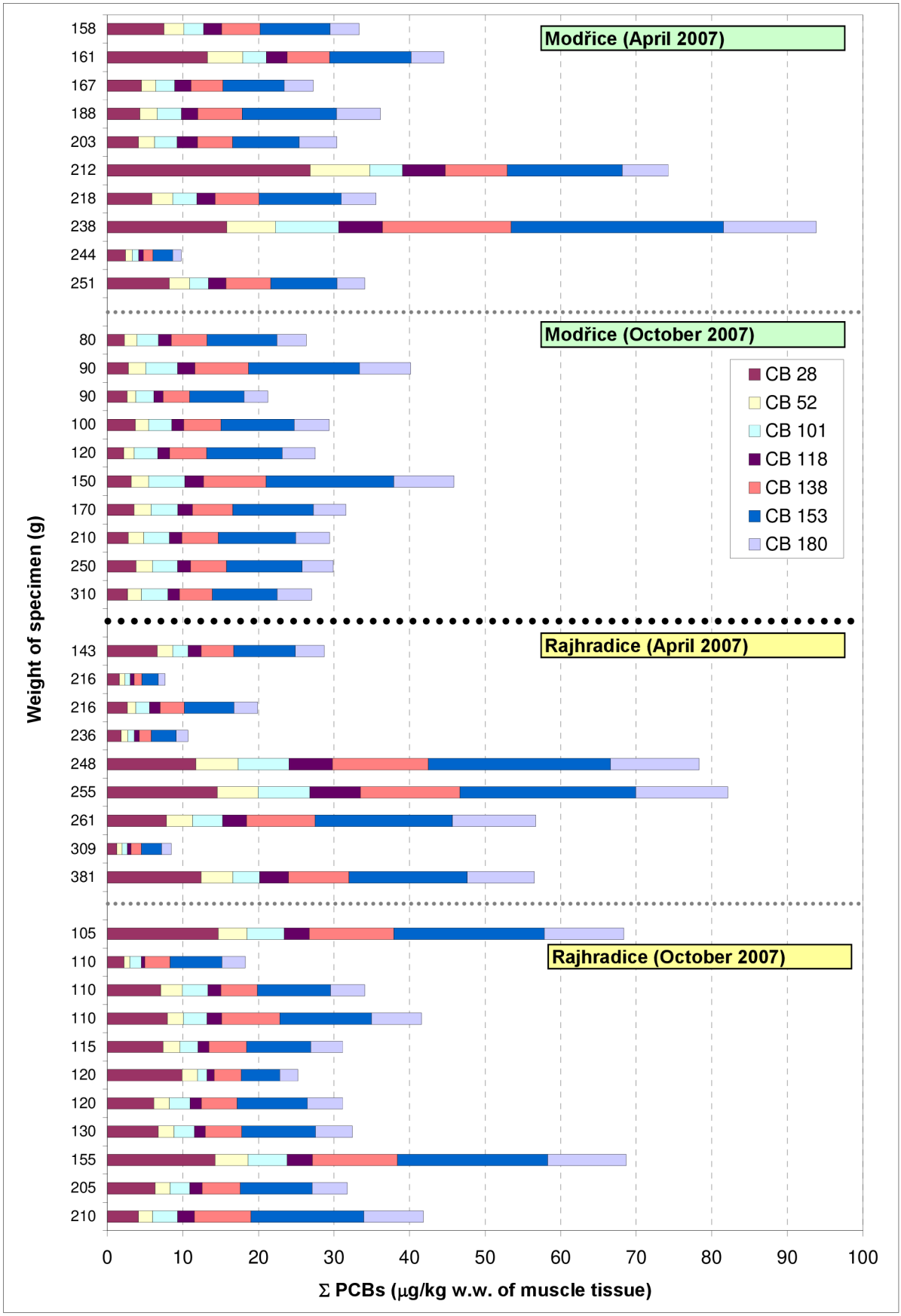


Fig. 42: PCBs in chubs from the Svratka River.

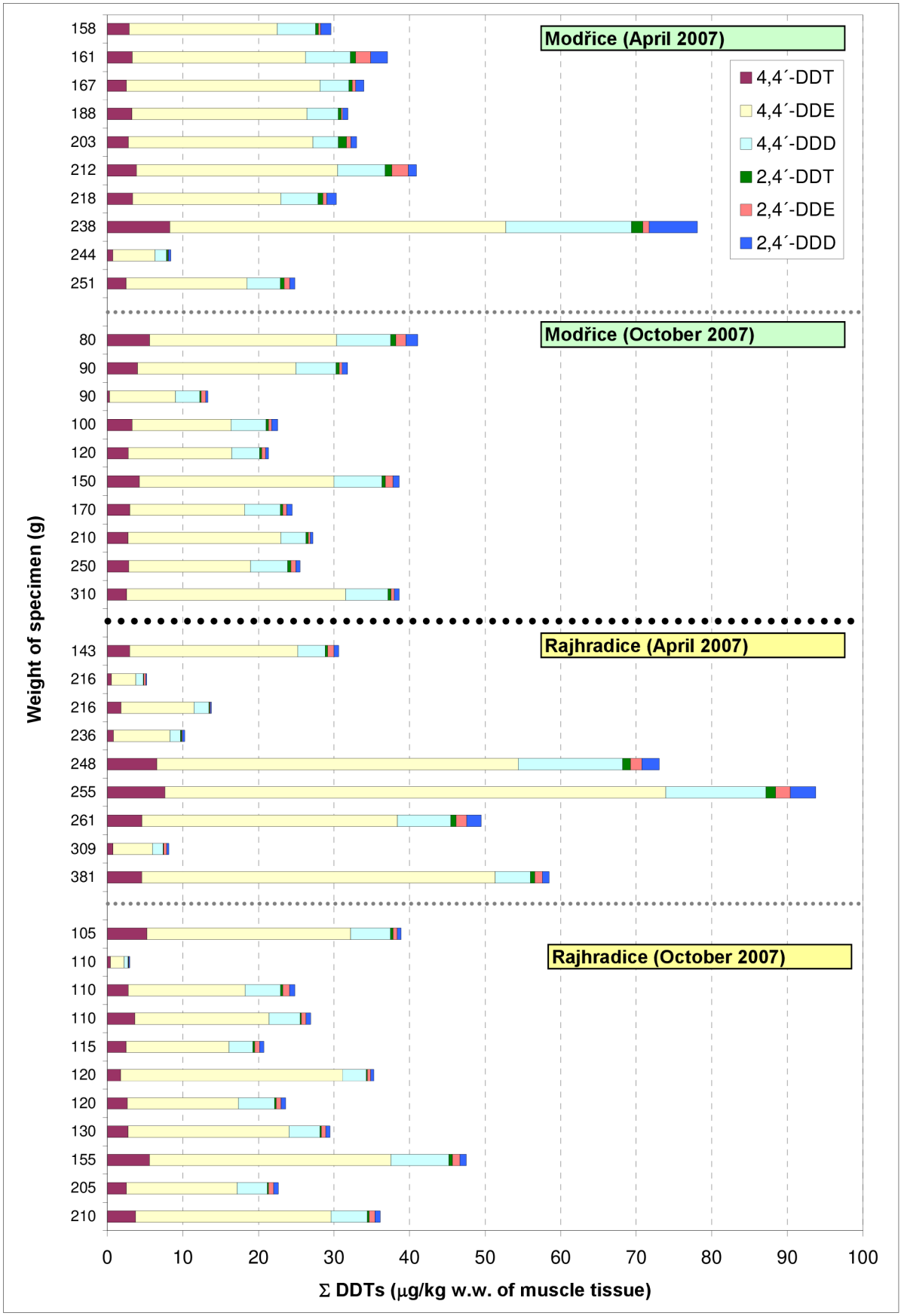


Fig. 43: DDTs in chubs from the Svratka River.

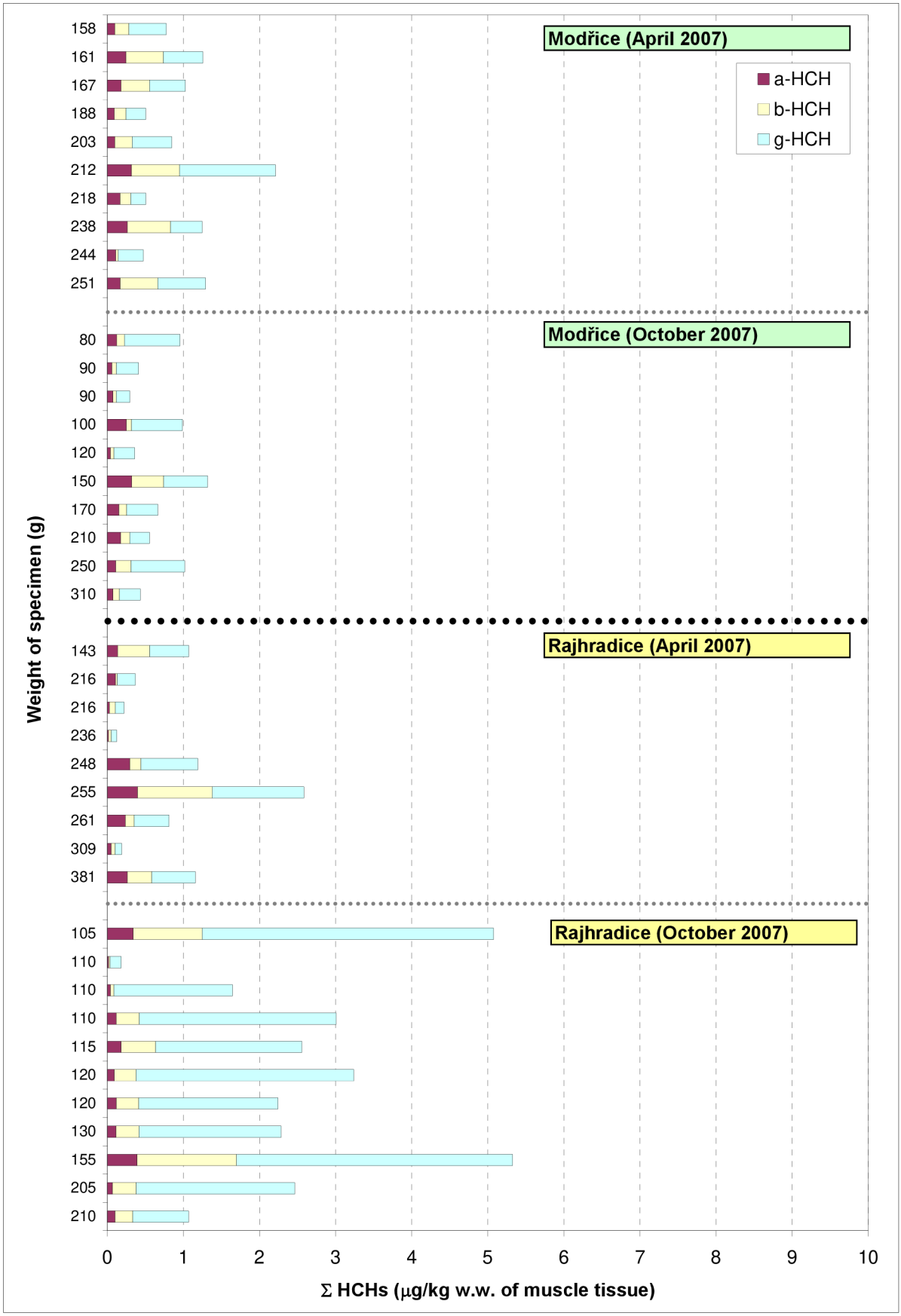


Fig. 44: HCHs in chubs from the Svratka River.

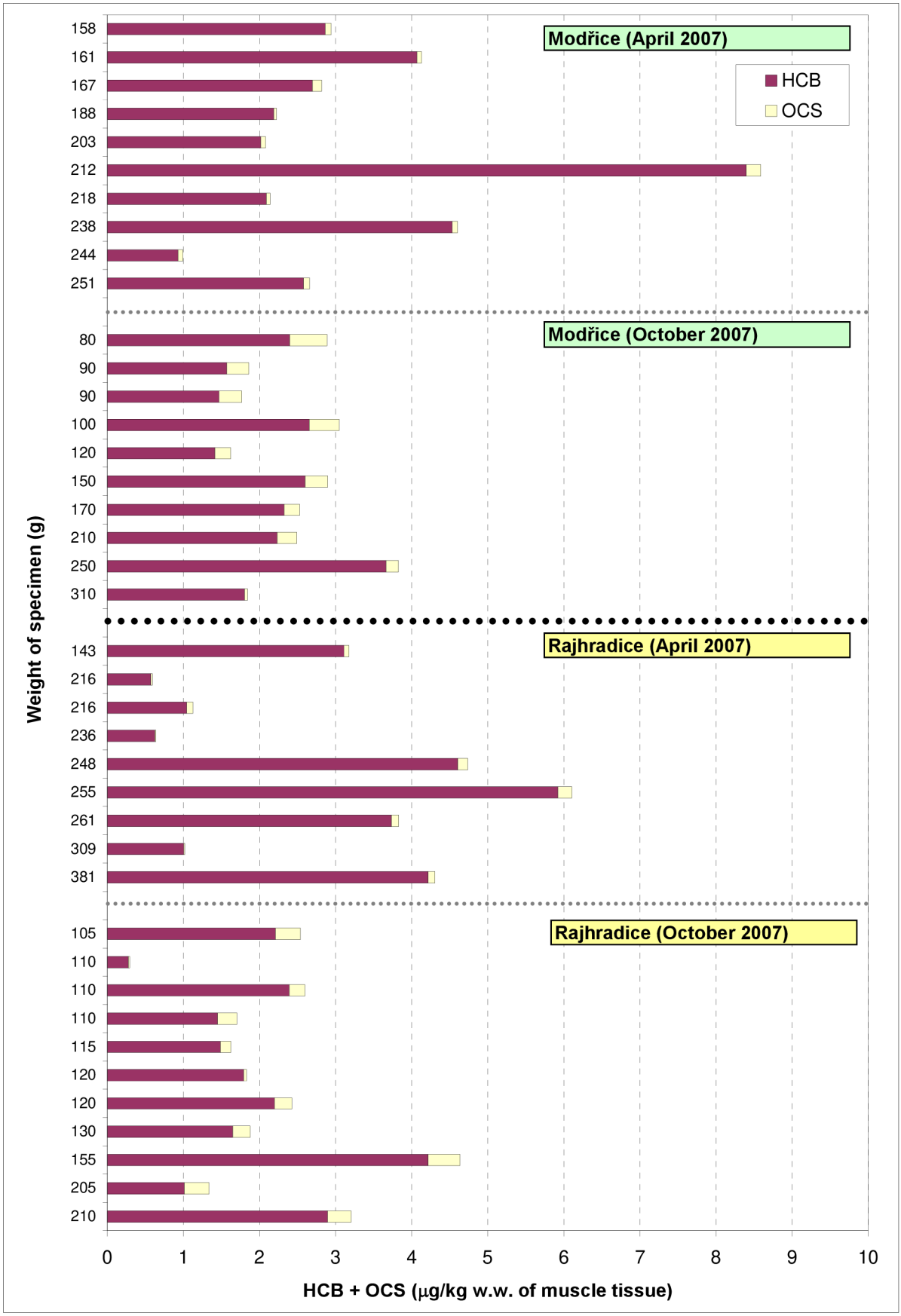


Fig. 45: HCB and OCS in chubs from the Svratka River.

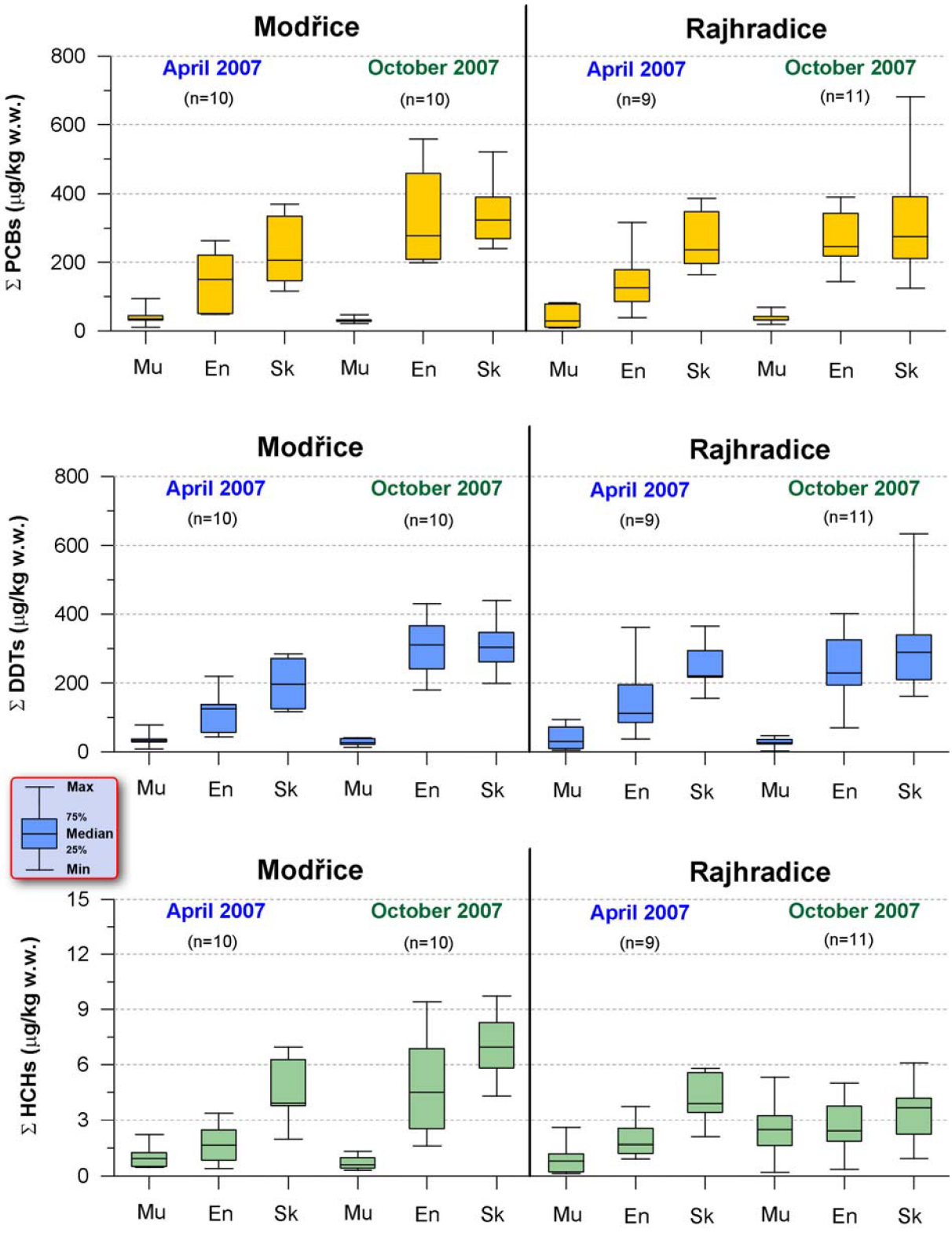


Fig. 46: Levels of PCBs, DDTs and HCHs in selected tissues of chubs from the Svratka River.

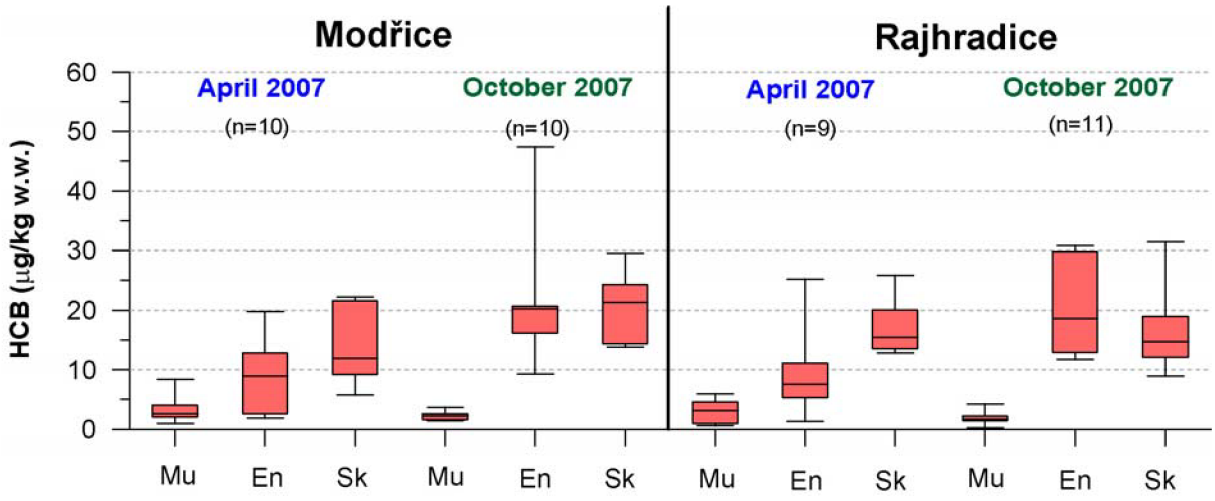


Fig. 47: Levels of HCB in selected tissues of chubs from the Svratka River.

Considering the hygienic limits quoted in the Decree of the Ministry of Health of the Czech Republic No. 381/2007 and 306/2004 Coll. (2,000 µg/kg ww for PCBs, 500 µg/kg ww for DDTs, 50 µg/kg ww for HCB and 20 µg/kg ww for γ-HCH), the levels in chub from this study were by far lower than the limits. We assume that even older fish of greater weights would not near these values.

When compared to other similar findings (not only) from the Czech Republic, the contamination of chub from the Svratka River with organochlorine pollutants seems not to differ from the common background levels. Chub from the River Labe, caught during 2001-2003, showed averages levels of PCBs from 16.7 to 106 µg/kg ww for similar weight categories (115-410 g of bw), the values of DDTs ranged from 15.5 to 39.8 µg/kg ww and of HCB ranged from 0.5 to 6.8 µg/kg ww. However, the maximum levels measured in 2003 were significantly affected by disastrous floods during 2002. The levels of PCBs in chub from the River Vltava were similar ranging from 23.9 (in 2002) to 100 (in 2003) µg/kg ww [**263**]. Chub from the River Morava in 1996 and 1998 had the average levels of PCBs of 23.1 and 18.2 µg/kg ww and DDTs had the mean values of about 11 and 19.2 µg/kg ww for the same weight category [**264**]. Low levels of PCBs and DDTs (4.97 and 0.4 µg/kg ww, resp.) were determined in chub from the River Nestos, Greece [**265**] and in similar omnivores like carp and roach (9.5 and 6.8 µg/kg ww for PCBs, 21.6 and 11.4 µg/kg ww) from the Danube Delta [**127**].

In conclusion, the assumed differences between two sampling localities on the Svratka River, caused by e.g. local wastewater treatment plant, did not prove true and there were only tiny differences among the sets of samples. This was probably due to a relatively short distance between the sampling localities, despite the fact they were separated by a weir not allowing the fish to migrate. Moreover, short sampling period did not enable us to assess temporal trends in levels of POPs. The values did not exceed any hygienic limits for freshwater fish and represented usual background levels in the Czech Republic.

5.1.4. Organochlorine pollutants in fish from recreational fishing

Recreational and sport fishing has been one of the most popular leisure time activities (not only) in the Czech Republic for decades. Amateur fishers catch about 4.5 thousand tons of fishes from 42,000 hectares of ponds and reservoirs every year [**248**]. However, the ubiquitous environmental contamination with persistent organic pollutants affected the quality and hygiene safety of wildlife including fishes and thus an increased attention must be paid to monitoring and control the levels of pollutants in fish species as well as in their living environment. There have been several monitoring programmes and surveys focused on the determinations of POPs in marketable carp as the main freshwater fish species in the Czech Republic [**223**, **249**].

This study was aimed at the determination of organochlorine pollutants in fishes caught by an amateur fisher at various localities during 2007, and at the evaluation of risks from their consumption. The sampling localities were chosen randomly and the common carp together with one specimen of the bream were the species analysed. All samples of edible tissues (pooled samples of muscle and skin) were analysed for the content of polychlorinated biphenyls and selected organochlorine pesticides.

Concentrations of ΣPCBs in pooled samples of edible tissues (muscle and skin) of the fishes investigated are presented in **Figure 48**. Mean value of ΣPCBs was 44.7 μg/kg wet weight (ww) and the range from 7 to 116 μg/kg ww. However, as pooled samples of muscle and skin were analysed, higher lipid content in the samples affected the values of POPs and we suppose them to be lower in case only muscle would be processed. On the other hand, fishes are mostly consumed together with skin the high fat content of which comprises substantial amount of POPs. Considering the possible differences in PCB levels among the four sampling localities, the data seemed not to show any significant differences. However, small number of specimen and their heterogeneity (different weights and ages) did not allow any statistical comparison. Moreover, the pooled samples of tissues were of varying lipid content, which might distort the results. And finally, an important factor influencing the results could be the fact that some of the caught specimens were not grown in the sampled pond/reservoir. At least one of the ponds is commercial water (Čertův pond in Čunín or Sobáčov), i.e. the fishes do not grow up from spawn there, but adult specimens of sizes above legal limits are planted so that they can be directly caught by fishers for charge. Patterns of PCB congeners in the fishes seem to prove this assumption. For instance, the two carps from Čunín showed quite different relative abundances of PCB congeners. The first one (CA03) had PCB 28 and 52 accounting for 75%, compared to the second specimen (CA06) with only 16.8%, which was surprising. Another example could be the locality of Tovačov Lakes where the relative contributions of PCB 28 and 52 ranged from 31.9% to 63.2%.

The levels of ΣDDTs in the fishes ranged from 6.1 to 242.3 μg/kg ww with the mean value of 85.0 μg/kg ww (**Figure 49**). The *p,p′*-DDE metabolite was the most abundant compound accounting for 39.7-82.1% of total DDTs and followed by *p,p′*-DDD which accounted for 11.3-34.5%. The abundance of the parental *p,p′*-DDT was low with the mean contribution of 6.5% (ranging from 0.8% to 17.8%) which suggest, together with predominance of *p,p′*-DDE (the average DDE/DDT ratio was 0.13), that the current emissions of DDT are relatively low. There was significant correlation between the levels of PCBs and DDTs in lipids of the samples ($n = 10$, $r = 0.861$, $p < 0.01$). And again, there were no relationship between the levels of POPs and the sampling locality, probably due to the reasons described above.

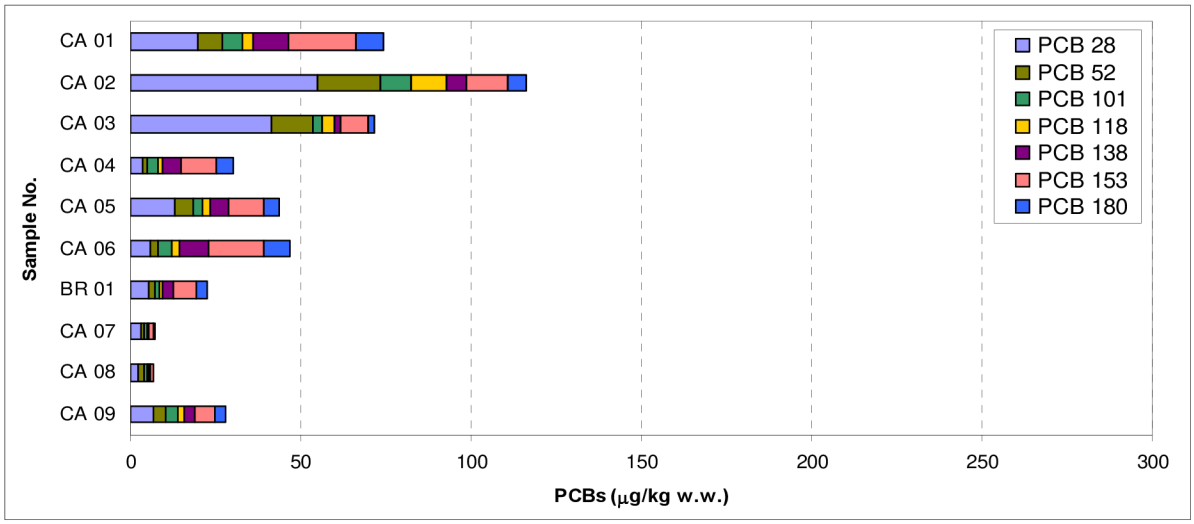


Fig. 48: Levels and relative abundances of PCBs in fishes from recreational fishing.

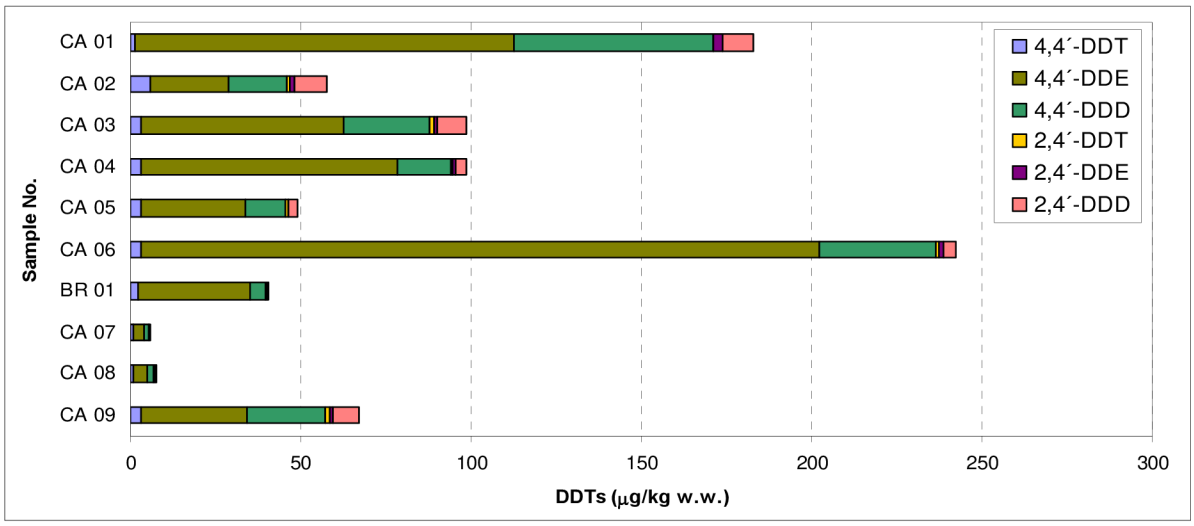


Fig. 49: Levels and relative abundances of DDTs in fishes from recreational fishing.

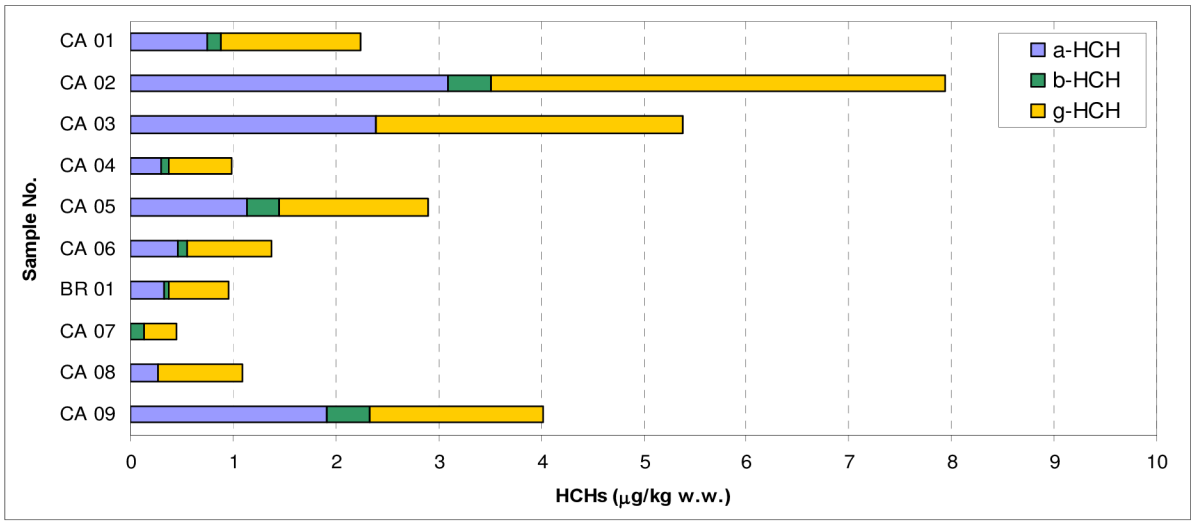


Fig. 50: Levels and relative abundances of HCHs in fishes from recreational fishing.

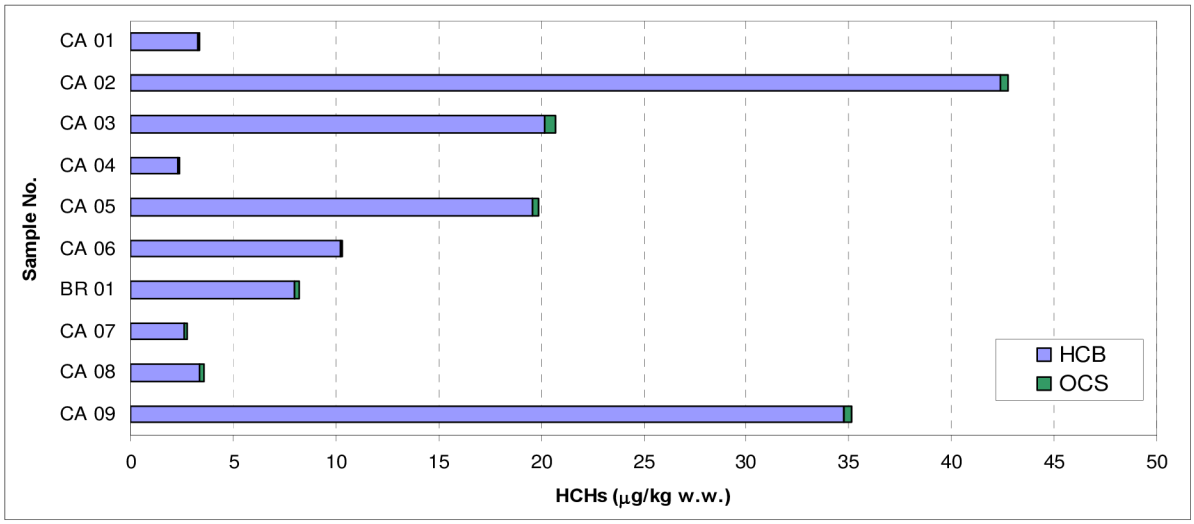


Fig. 51: Levels and relative abundances of HCB and OCS in fishes from recreational fishing.

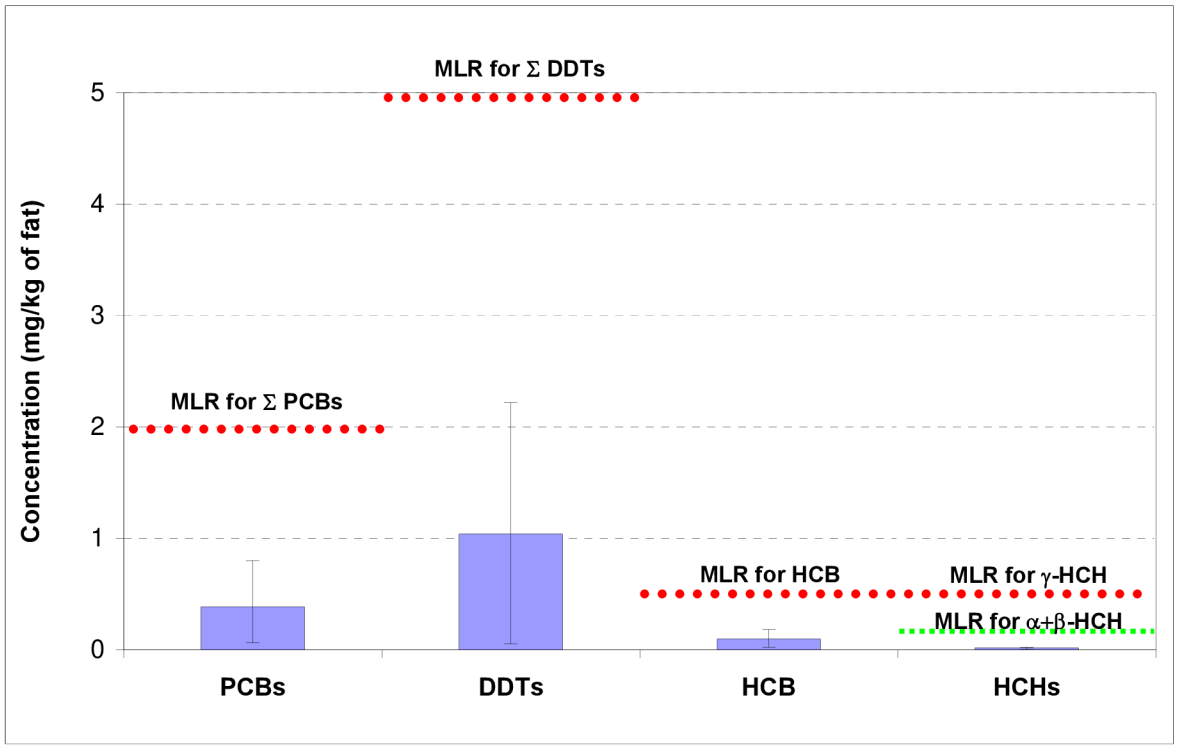


Fig. 52: Mean concentrations of organochlorine contaminants in fishes from recreational fishing compared with relevant values of maximum residual limits (MRL). Error bars represent 10% and 90%.

The contents of HCHs in samples were substantially lower than those of PCBs and DDTs and they did not exceeded 10 μg/kg ww, as can be seen in **Figure 50**. The range of concentrations was from 0.46 to 7.9 μg/kg ww, with the mean of 2.7 μg/kg ww. The proportion of individual HCH isomers were in order γ-HCH > α-HCH >> β-HCH. The β-HCH was not detected in two samples and α-HCH was missing once. HCHs were positively correlated with both PCBs ($r = 0.766$, $p < 0.01$) and DDTs ($r = 0.507$, $p < 0.01$).

Shown in **Figure 51** are the concentrations of HCB and OCS that are unintended by-products of various industrial processes. The sums of HCB and OCS were found to be in range from 2.4 to 42.8 μg/kg ww, the mean value was 14.9 μg/kg ww. However, HCB was by far more abundant compound accounting for about 97%. HCB poorly correlated with both PCBs ($r = 0.313$, $p < 0.01$) and DDTs ($r = 0.302$, $p < 0.01$).

The ADI (Accetable Daily Intake) values determined by WHO for PCBs, DDTs and HCB are 5, 20 and 0.6 $\mu g.kg^{-1}$ of body weight per day, respectively. These values can be used for assessment of health risks from consuming contaminated food and tell us how much of particular contaminant can be 'consumed' per day with no risk. Comparing the highest levels of POPs determined in the samples to the above values (common body weight taken as 70 kg) one should consume about 3 or 5.8 kg of carp flesh daily to reach the ADI for PCBs or DDTs, respectively. However, these values would be probably higher because the samples analysed included skin whereas in other cases only levels of POPs in muscle tissue are determined and compared. Taking the annual freshwater fish consumption in Czech Republic into consideration (about 1.2 kg per capita, i.e. 3.3 g a day [**250**]), the dietary intake of PCBs and DDTs from the considered fish would account for about 0.11% and 0.06% of the ADI values, resp. The risks are thus negligible.

We can also compare the results with the values given in the Directives of the Czech Ministry of Health No. 381/2007 Coll. and 306/2004 Coll. that set the maximum limits of contaminants and pesticide residues in foodstuff. Since the lipid content in the samples was higher than 10% (because of mixed tissue samples), the limits for DDT, HCB and HCH are ten fold higher then the values given by the regulation. As can be seen in **Figure 52**, the values of PCBs and organochlorine pesticides did not exceeded the given limits and they were several times lower in most samples.

Svobodová et al. published levels of POPs in marketable carps from the region of South and West Bohemia (Czech Republic) in 2003 and 2004 [**223**, **251**]. They reported comparable concentrations of PCBs and organochlorine pesticides, which did not exceed or even reach the discussed limits. Means of ΣPCBs ranged from 4.52 to 98.85 μg/kg ww and the PCB 138, 153 and 180 were the most abundant congeners in most carps. ΣDDTs in carps ranged from 7.91 to 86.48 μg/kg ww and the *p,p'*-DDE metabolite was the dominate compound in most samples. HCHs, HCB and OCS levels were in range of 0.2-6.33 μg/kg ww, 0.31-6.17 μg/kg ww and 0.02-0.8 μg/kg ww.

To conclude this chapter, the investigated fishes did not show any significant load with organochlorine pollutants and the consumption of them did not pose any serious health risks. Moreover, the data from this study (despite the fact mixed tissue samples were analysed) are comparable with other findings in the Czech Republic and seem to represent general values of POPs in this fish species.

5.2. Needles as a suitable tool for the monitoring of POPs in the environment

Despite many efforts towards bans or restrictions on the use of organochlorine compounds as PCBs or DDT during past decades, these keep on polluting the global environment and can be still found in almost all environmental samples. One of the common methods used for the assessment of the environmental load with persistent organic pollutants is direct determination of their concentrations in samples of terrestrial flora including e.g. needles, mosses or leaves. Such 'passive bio-samplers' serve well as bioaccumulation markers for they are able to accumulate contaminants over a long time from the ambient air and can be used to monitor atmospheric pollution on global, regional and local scales. What is more, they are easier to collect than passive air samplers. Needles of coniferous trees belong to the most analysed samples and there have been plenty of papers dealing with this issue published so far (findings from selected studies presented in **Table 21**).

Within this study, needles from three coniferous tree species were collected during the years 2006 and 2007 and mixed samples of one- and two-year needles were taken. The sampling localities represented both relatively clean rural areas and urban and industrial sites. All samples of needles were analysed for the content of polychlorinated biphenyls and selected organochlorine pesticides (findings summarised in **Figure 57**).

The levels of ΣPCBs in the needles investigated and relative abundances of individual PCB congeners are shown in **Figure 53**. In all samples the concentrations of one or more PCB congeners were determined as below the limit of quantification (the values were then expressed as half the LOQ value, here 0.025 ng/g ww), some of them were not detected in several samples. Therefore, total PCB concentrations were in the range from <0.15 to <0.82 ng/g ww in pine needles, from <0.45 to <1.0 ng/g ww in fir needles and from <0.2 to <0.91 ng/g ww in blue spruce needles. Mean levels of PCBs in samples from different conifers were alike: 0.45 ng/g ww (pine), 0.70 ng/g ww (fir) and 0.60 ng/g ww (blue spruce). As can be seen in **Figure 53**, there were no significant differences, both interspecies ($p < 0.001$) and intraspecies. And what can be learnt more from the results, no differences were found between the group of samples from rural and urban areas. PCB congener No. 28 was the most abundant in all samples accounting for (on average) 54.7%, 44.6% and 49.2% of the ΣPCBs in pine, fir and blue spruce needles, respectively. The relative abundances of the other PCB congeners were in the order CB 153>CB 52>CB 138> >CB 118>CB 180>CB 101. They contributions to the ΣPCBs ranged from 13.4% to 4.6% for PCB 153 and PCB 101, respectively. However, these congeners were below the LOQ value in many samples.

Shown in **Figure 54** are the levels of ΣDDTs in the investigated samples of needles. DDTs were found in the samples at lower concentrations than PCBs and their mean levels were 0.16 ng/g ww (pine), 0.38 ng/g ww (fir) and 0.25 ng/g ww (blue spruce). The highest concentrations of ΣDDTs were measured in two samples of fir needles (0.73 and 0.75 ng/g ww in samples No. 10 and 11, resp.). However, including these two samples of fir needles, no significant differences were found among the samples ($p < 0.001$). The parent compound, *p,p'*-DDT, was the most abundant DDT isomer in fir and blue spruce needles accounting for 37.8% and 34.5% of ΣDDTs, respectively. The aerobic metabolite of DDT, *p,p'*-DDE, dominated with 27.8% of ΣDDTs in pine needles. The contributions of both *p,p'*-DDT and *p,p'*-DDE to total ΣDDTs were nearly equal in all conifer species. The other isomers and metabolites of DDT including *p,p'*-DDD were below the LOQ value in most samples,

o,p'-DDD was not found in only a few samples. Again, no differences were found between the group of samples from rural and urban areas.

The concentrations of ΣHCHs (α-, β- and γ- ismomers) determined in the needles are shown in **Figure 55**. The average values of ΣHCHs were 0.12 ng/g ww (pine) and 0.15 ng/g ww (both fir and blue spruce). The α+γ-HCHs dominated in almost all samples and accounted for 62.2-100% of total HCHs. Their ratios (α-/γ-HCH) ranged from 0.11 to 3.48. The average values of α-HCH and γ-HCH in all samples were 0.03 and 0.1 ng/g ww, resp. γ-HCH had more findings above the LOQ than the α-isomer. On the contrary, β-HCH had a positive detection only in 6 out of 23 samples. However, five of these findings were below the LOQ. Higher levels of γ-HCH may come from the use of the pesticide 'lindane' which is composed of pure γ-HCH isomer and its application reduce the ratio of α-/γ-HCH in the environment. On the other hand, α-HCH tends to stay in the environment for longer time. Thus, current concentrations and the ratios of HCH isomers might be the combined results of different HCH sources from the past and present time.

The last pollutant determined in the needle samples was HCB and the values found are shown in **Figure 56.** Except one locality with outstanding concentration of HCB (0.46 ng/g ww, pine needles, sample No. 1), the levels of HCB were somewhat low in comparison to the other pollutants measures. Mean levels of HCB in samples were 0.11g/g ww in pine needles, 0.06 ng/g ww in fir needles and <LOQ in blue spruce needles.

Comparison with some other findings is presented in **Table 21**. As can be seen from the data, the levels of organochlorines in needles from the Czech Republic are on average lower than those from other countries. For instance, Holoubek et al. [**231**] published the results of POP monitoring in needles from the Košetice background region (Czech Republic) and compared them to some other foreign studies. He reported relatively low levels of POPs determined in pine needles during 1988-94 in the Košetice region. The concentrations of PCBs (No. 28, 52 and 101) and *p,p'*-DDT and *p,p'*-DDE were <0.1 ng/g in all samples, while the means of HCB, α- and γ-HCH were 2.63, 0.22 and 1.98 ng/g ww, respectively. Similarly, Kocourek et al. [**239**] reported comparable values of 0.67 ng/g ww for ΣPCBs, 0.65 ng/g ww for ΣDDTs, 0.84 ng/g ww for ΣHCHs and 1.07 ng/g for HCB (data from 2000). Both studies considered these findings to be much lower than those from other countries, where the levels were one or two orders of magnitude higher. Moreover, Kocourek et al. reported a slightly declining trend in the concentrations of PCBs from 1.87 ng/g in 1995 to 0.67 ng/g in 2000. The ratio of *p,p'*-DDT/*p,p'*-DDE in their study did not vary, the levels of *p,p'*-DDD metabolite were much lower compared to the two other DDTs and there was a decrease in concentrations of DDTs observed from 1995 to 2000. Their findings from 2000 correspond with those from this study. But what is different, in their samples, high chlorinated PCB congeners accounted for majority in the sum of PCBs and they found higher concentrations of HCB (approx. ten times higher levels) which were similar to the findings of Holoubek et al. (<0.1-6 ng/g ww, mean value of 2.63 ng/g ww).

There were no clear patterns or fingerprints in the ratios/concentrations of the monitored pollutants that could be related to specific pesticide/compound. Unlike other countries where particular compound (e.g. α-HCH in Finland, *p,p'*-DDT in Italy and China) dominated in needles and pointed at a concrete source of pollution [**231**, **234**, **235**], in the Czech Republic, however, the present patterns and ratios of individual contaminants are rather 'a mixture' of compounds from formerly applied products and current local and transboundary emissions.

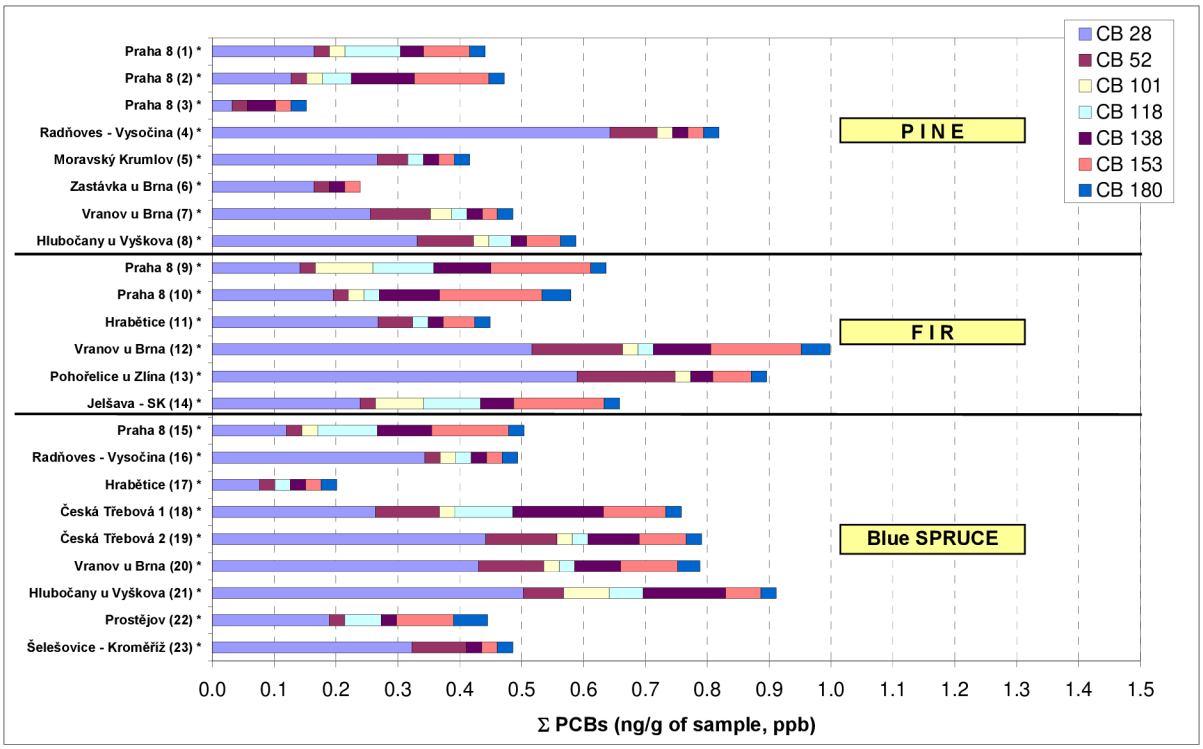


Fig. 53: Levels and relative abundances of PCB congeners in needles of three conifer species from the Czech Republic.

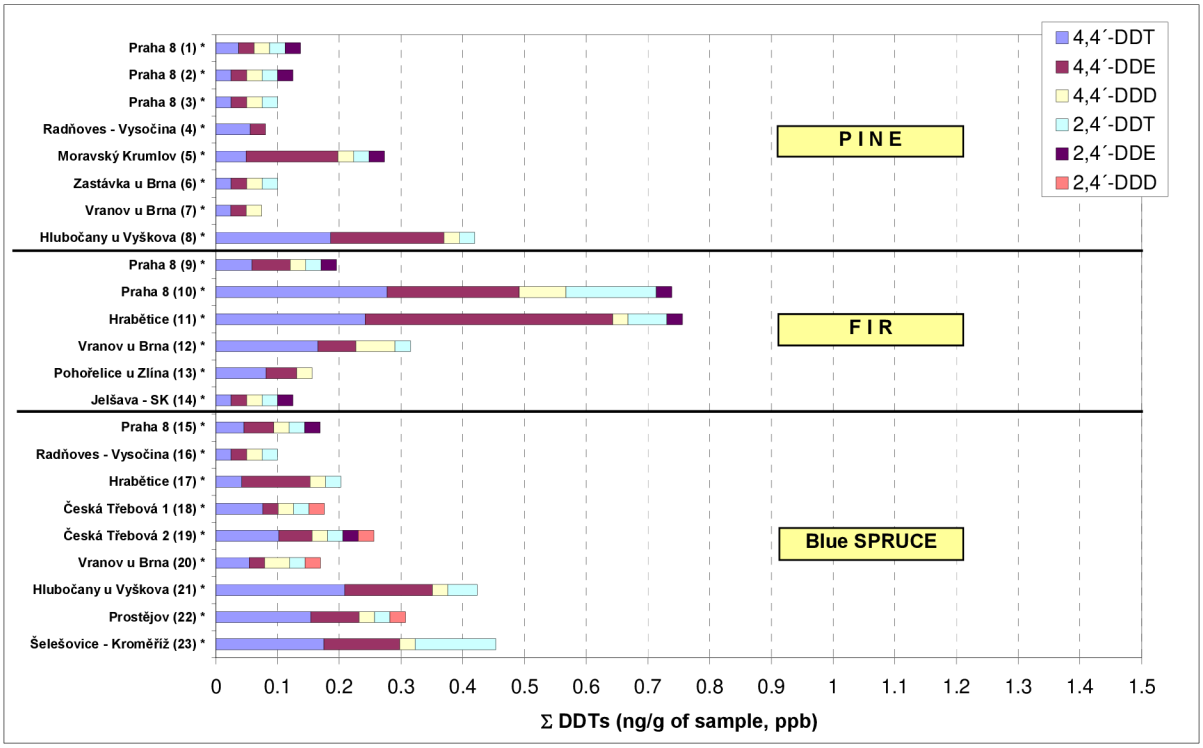


Fig. 54: Levels and relative abundances of DDTs in needles of three conifer species from the Czech Republic.

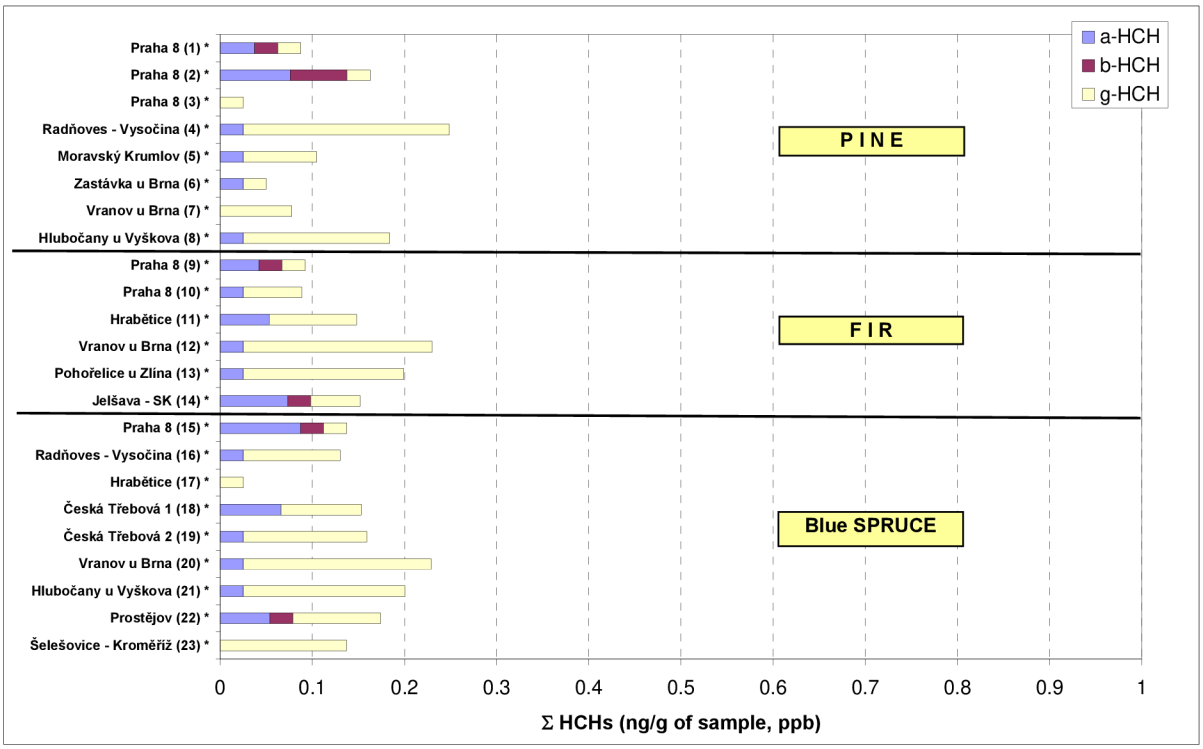


Fig. 55: Levels and relative abundances of HCHs in needles of three conifer species from the Czech Republic.

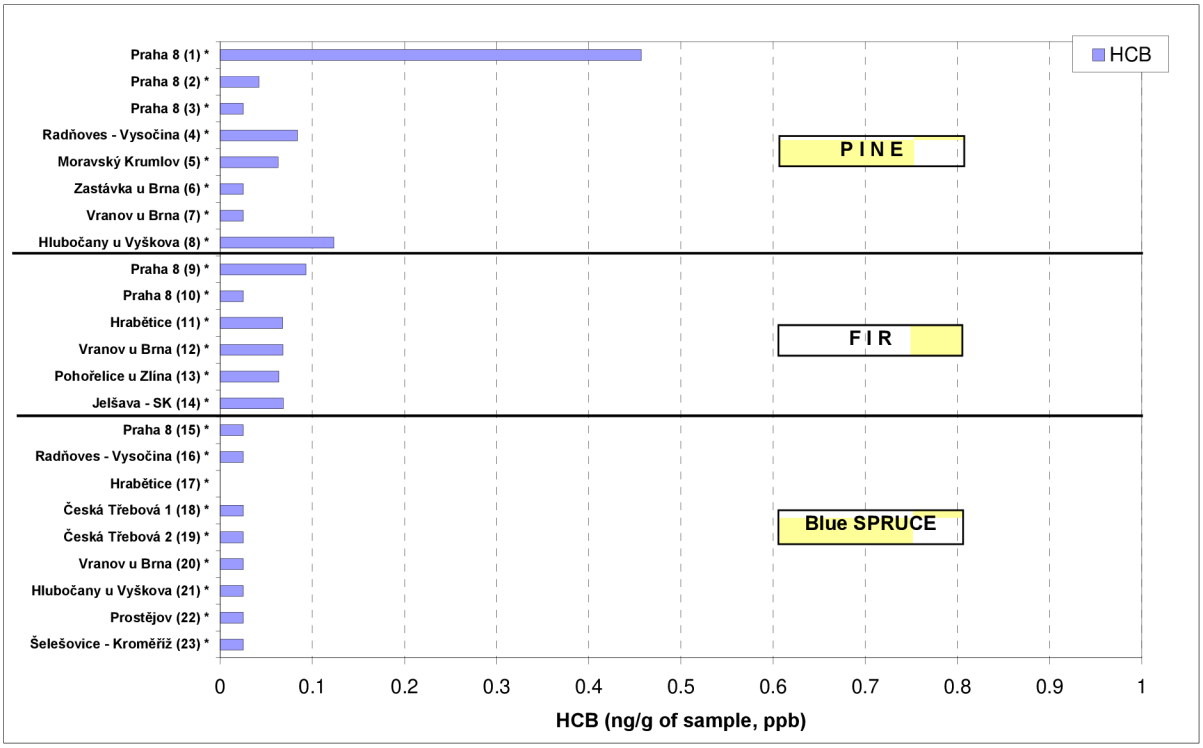


Fig. 56: Levels and relative abundances of HCB in needles of three conifer species from the Czech Republic.

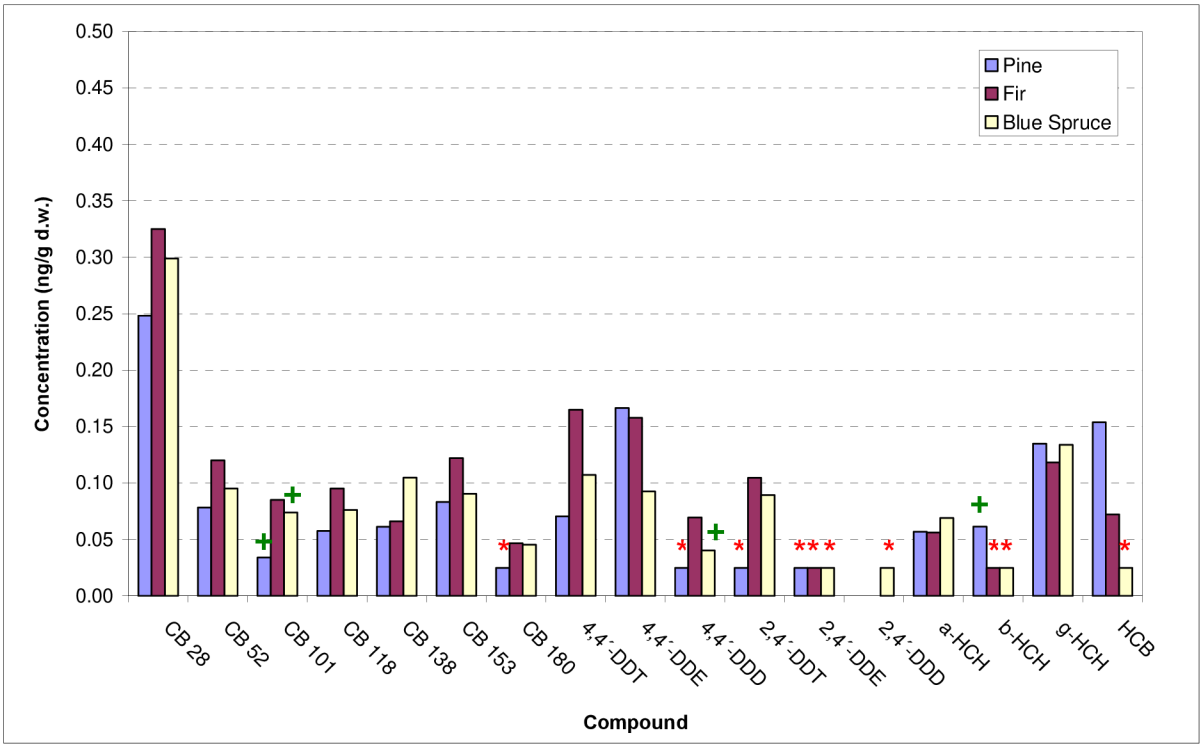


Fig. 57: Mean concentrations of monitored pollutants in needles of three conifer species. * - all values below LOQ, + - the only value above LOQ

Tab. 21: Organochlorine pollutants in needles from different countries (ng/g)

Country (year of sampling)	**ΣPCBs**	**ΣDDTs**	**ΣHCHs**	**HCB**	**Ref.**
Czech Republic (1988-94)	<0.1[a]	<0.1[b]	<0.1-9.2[c]	<0.1-6	**[231]**
Czech Republic (1995-2000)[f]	1.87-0.67	2.16-0.65	1.13-0.84	0.15-1.07	**[239]**
Sweden (1991-94)	1.0-3.0	-	-	-	**[238]**
Croatia (1998)[e]	3.2-30.07	0.51-12.05	0.4-18.56	0.14-2.81	**[233]**
Croatia (2006)[e]	5.66-16.82	0.69-1.98	3.09-10.06	0.36-1.70	**[236]**
Poland (2002)	2.8-50	-	-	-	**[232]**
Japan (1999)	5.1-73				
China (2001)	-	nd-30.9	4.7-51.5	nd-11.7	**[234]**
China - Beijing (2002)	8.8-270.5	12.5-113.3	11.7-20.8	nd-12.6	**[235]**
Tibetan Plateau (2006)	-	1.9-20.5	0.39-4.9	0.69-4.3	**[237]**

[a] CB 28+52+101; [b] DDT+DDE; [c] γ-HCH; [d] Sum of Kanechlors 300, 400, 500 and 600; [e] two-year-old needles; [f] average PCB levels in 1995 and 2000

Moreover, fairly low values of POPs determined in needles (many of them just above the LOQ value), together with a relatively small number of the samples investigated within a short period of time, do not allow any more comprehensive assessment of the results. Nevertheless, the data obtained in this study are in good accordance with other findings and proves needles to be a suitable tool for direct determination of environmental pollution.

5.3. Tissue-specific distribution of organochlorine pollutants in birds of prey

Birds of prey, just like other top predators, are highly susceptible to the accumulation of persistent pollutants due to the fact they are located at the top of the food chain (for more details and references, see pp. 36). This fact, together with their slow reproduction rates and low enzyme activities to degrade organochlorine compounds, makes them a very sensitive biomarkers of environmental quality. Our aim was to assess the current levels of chlorinated POPs in tissues of selected raptor species from the Czech Republic, to evaluate the distribution of POPs among individual tissues of cormorants, to prove the usefulness of bird feathers as a non-destructive biomonitoring tool for organic pollutants and to evaluate the process of bioaccumulation of POPs in an aquatic food web.

Bioaccumulation of organochlorines in aquatic biota a raptors from the Záhlinice Ponds

Sediment and specimens of six fish species and three raptor species were investigated in order to assess the process of bioaccumulation of POPs within an aquatic food chain.

Shown in **Figure 58** are mean concentrations of organochlorine pollutants in sediment and fishes from the Záhlinice Ponds. Fishes investigated within this study came from another research carried out by Houserová et al. in 2003, focused on the determination of total mercury and mercury species in birds of prey and fish in an aquatic ecosystem in the Czech Republic. Descriptions of species investigated can be found in [**246**]. Briefly, the fishes caught were of low range of age (2-4 years) and hence the age should not affect the levels of POPs in their tissues, which is mainly influenced by their diet and living environment. ΣPCBs (in this chapter sum of only 7 indicator CB congeners are considered for all samples) and ΣDDTs were the most abundant compounds in both sediment and fish tissue samples. In sediment, the levels of ΣPCBs and ΣDDTs were 24 and 38 μg/kg dry weight, respectively, and on average these levels did not vary ($p < 0.01$) from those found in tissues of the fishes investigated. Contrarily, the concentrations of HCB and ΣHCHs in both sediment and fishes were much lower – mostly one order of magnitude differences compared with the ΣPCBs and ΣDDTs levels. And again, the concentrations of HCB and ΣHCHs did not significantly differ ($p < 0.01$) from those found in fishes. Seemingly, no clear conclusion can be drawn from the levels of POPs in fishes for the interspecies differences do not exactly follow their feeding behaviour (e.g. predatory pike versus omnivorous common carp, **Figure 58**). However, these values are distorted by different content of fat in fishes. When non-lipid-standardised values are compared, expected higher burdens of predatory fishes with POPs prove true. For instance, concentration of ΣPCBs in tissue of predatory pike was determined as 1628±252 μg/kg lipid weight (lw), whereas in omnivorous common carp or in herbivorous grass carp, it was only 478±241 and 225±17 μg/kg lw, respectively. A leap in concentrations of POPs can be observed between fishes and birds of prey. Means of ΣPCBs and ΣDDTs in muscle tissue of cormorants and grey herons from the Záhlinice Ponds were 102±146 and 70±63 μg/kg ww, with maximum values of 455.7 μg/kg ww of ΣPCBs and 660.4 μk/kg ww for ΣDDTs (both in cormorants). A dietary pyramid based on the data measured is drawn in **Figure 61** and shows changes of mean concentrations of ΣPCBs from 'abiotic' sediment (24 μg/kg dw) positioned at the very beginning of the food chain to its top represented by cormorants and herons. As for the other contaminants monitored, HCB and Σα+γ-HCHs showed no changes in concentrations from sediment (4.2 and 2.1 μg/kg dw for HCB and ΣHCHs, resp.) to fishes (average values 3.2 and 5.1 μg/kg dw for HCB and ΣHCHs, resp.). In birds of prey, the values

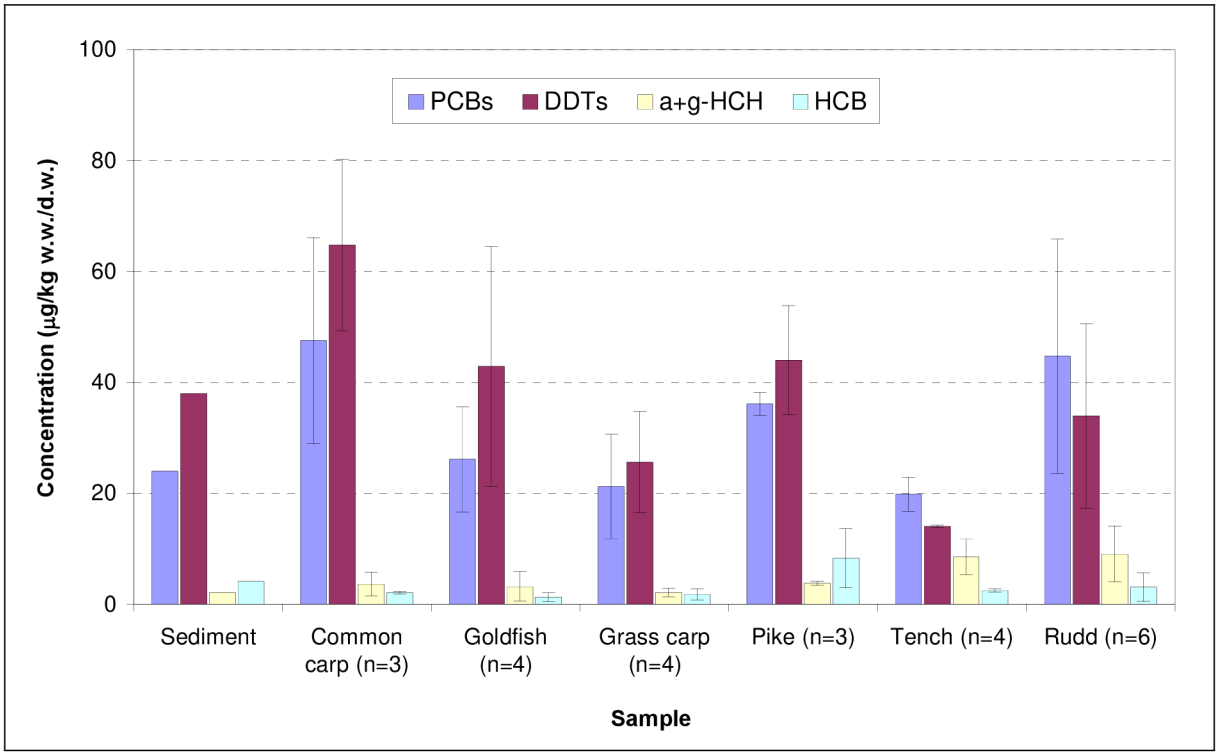


Fig. 58: Mean concentrations of organochlorine pollutants in sediment and fishes from the Záhlinice Ponds. Error bars represent SD.

seemed not to be significantly higher. Mean levels of HCB and ΣHCHs in cormorants were 5.1 and 1.1 µg/kg ww, resp., and mean levels in herons were 7.3 and 0.3 µg/kg ww, resp. This data might suggest that α+γ-HCHs and HCB differ from the biomagnification pathways of PCBs and DDTs and do not increase their concentrations in the food web. For instance, Covaci et al. [**127**] published similar results for accumulation of POPs cormorants and aquatic biota from the Danube Delta, Romania (see **Table 22** for comparison). Their data showed a good correlation between log K_{ow} and biomagnification power (BMP) for PCBs, DDTs and PBDE 47. On the contrary, for HCB and α+γ-HCHs, no statistically significant correlation was observed. Low food web magnification of α+β-HCHs and the metabolisation of γ-HCHs by organism situated higher in the food chain have been published as well [**247**].

Relative abundances of individual PCB congeners and DDT metabolites and isomers in sediment and biota samples from the Záhlinice area are presented in **Figures 59** and **60**, respectively. Both groups of organochlorine pollutants showed obvious shifts in their patterns through the food chain. As for PCBs, the principal contributors to the ΣPCBs in sediment were PCB 28 and 52 accounting for 37.6% and 22.1%, respectively, while the PCB patterns in predatory heron were highly dominated by higher chlorinated and more persistent CB congeners 138, 153 and 180 (17.4%, 37.5% and 25.3%, resp.). Similarly, in sediment, the relative abundances of *p,p'*-DDE and *p,p'*-DDD were nearly identical (36.8% and 33.4%, resp.), whereas in cormorants and herons, the former metabolite was highly dominating (91.5% and 1.7% in herons and 94.3% and 0.4% in cormorants). Relatively low percentages of parental compound *p,p'*-DDT (12.5% in sediment, on average 9.1% in fishes and on av. 5.2% in raptors) may suggest there are no longer any significant sources of this insecticide. Moreover, *p,p'*-DDT is known to pose a shorter half-life in fish (~8 months) compared to that of *p,p'*-DDE and *p,p'*-DDD (~7 yrs) [**127**].

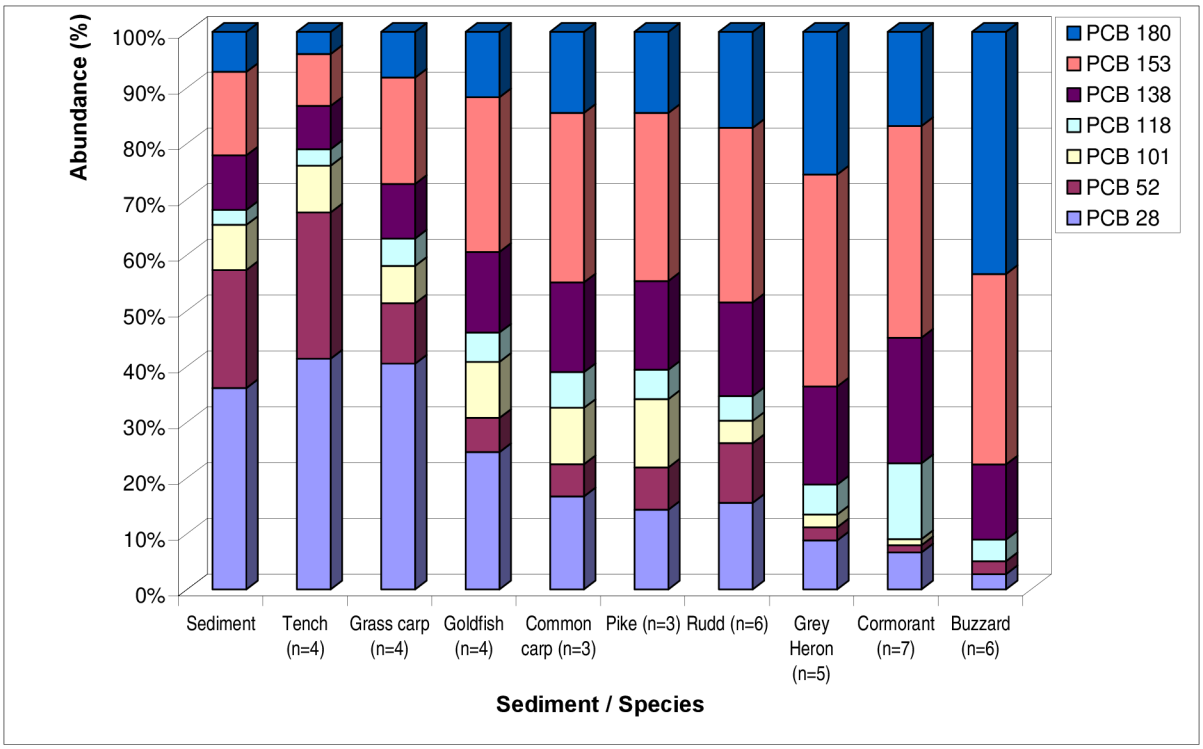


Fig. 59: Distribution of indicator PCB congeners in sediment and biota from the Záhlinice Ponds.

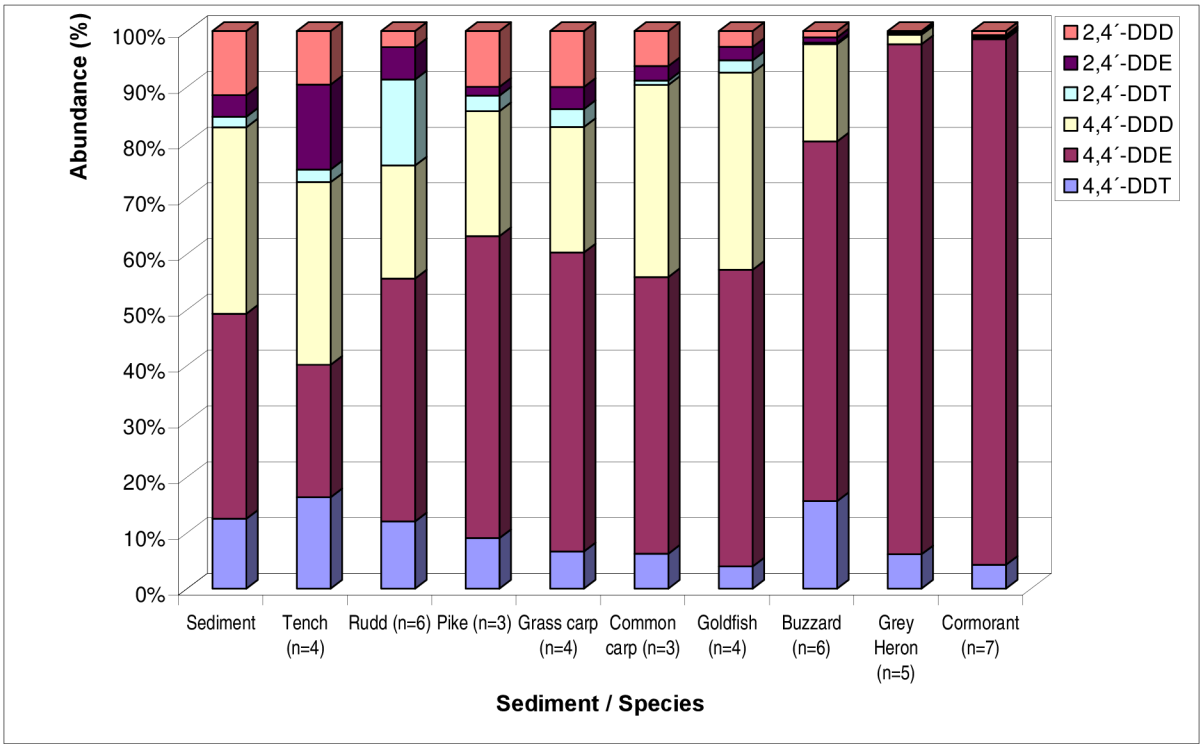


Fig. 60: Distribution of DDT metabolites and isomers in sediment and biota from the Záhlinice Ponds.

Relatively higher content of the anaerobic metabolite *p,p'*-DDD in some fishes as common carp or goldfish (34.5% and 35.3%, resp.) may be closely related to their higher feeding on benthos. Similarly, the same changes in patterns of PCBs and DDTs were found by Covaci et al. who showed that these compounds change their relative abundances towards the top of the food chain in favour of more persistent congeners and metabolites. However, this phenomenon has been known for decades and therefore our results are in good accordance with general trends. To complement the description of the data, buzzards from the Záhlinice Ponds were not included into this assessment since they have different feeding habits. However, the patterns of PCBs and DDTs in their tissues can serve for good comparison between terrestrial and aquatic raptors. For instance, a notable higher abundance of *p,p'*-DDD in buzzards can probably be associated to different POPs patterns in their prey (terrestrial species) compared to cormorants and herons (mainly aquatic predators). Moreover, higher chlorinated PCBs No. 138, 153 and 180 accounted for 90.1% of 7 indicator PCBs in buzzard, whereas in herons and cormorants it was only 80.1% and 76.5%, respectively.

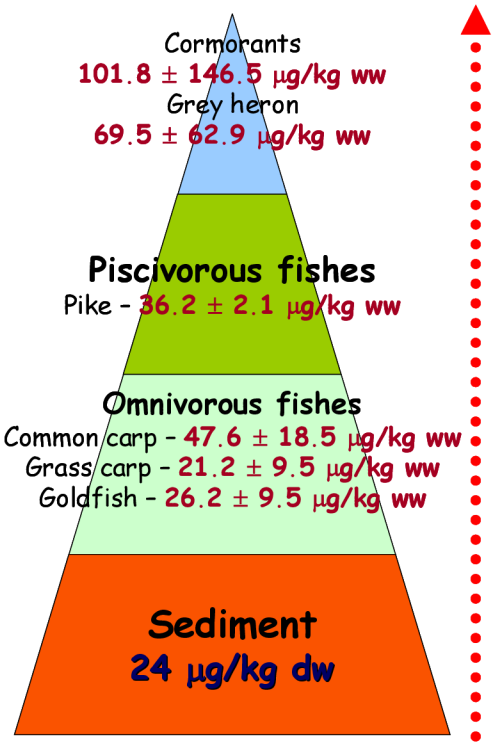


Fig. 61: Dietary pyramid from the Záhlinice Ponds food chain with the levels of Σ 7PCBs in sediment and muscle tissues of fishes and raptors (means ± SD)

To conclude this chapter, we can state that the analytical data obtained from the measurement of POPs in selected members of an aquatic food chain proved the anticipated bioaccumulation of persistent organic compounds, increase in their levels towards higher positions of the chain and last but not least the changes in patterns of selected organochlorines through this chain. The results are well comparable with similar foreign studies.

General comparison of levels of POPs in raptor species

Since the concentrations of POPs did not follow normal distribution and there were even several orders of magnitude differences, median values and ranges were used for further comparisons. Summary 'box-whisker' graphs with log-scaled y-axis showing medians, 1st and 3rd quartiles, and min/max values of ΣPCBs, ΣDDTs, ΣHCHs and HCB in pectoral muscle and liver tissues of three raptor species investigated are presented in **Figure 62.** As expected, the most abundant organochlorine compounds in both tissues of three studied raptor species were DDTs (in particular the aerobic metabolite *p,p'*-DDE) and PCBs, mainly the congeners with higher degree of chlorination. ΣHCHs and HCB were determined in raptor tissues in much lower concentrations as well as the other organochlorinated pesticides monitored, as discussed later.

The median values of ΣPCBs (22 congeners) ranged from 19.8 µg/kg ww in buzzards (ranging from 6.0 to 1157.8 µg/kg ww) to 130.9 µg/kg ww in grey herons (with a range of

4.1-3611.7 μg/kg ww). The median concentration of ΣPCBs determined in cormorants was 41.8 μg/kg ww (ranging from 4.4 to 867.1 μg/kg ww). As can be seen in **Figure 62**, there was at least one outlying value for each species that significantly differ from the other concentrations. High chlorinated PCB congeners No. 153, 138, 180 and 187 were the major constituents of total 22 PCBs in cormorants, accounting for 23.2%, 12.2%, 12.2% and 7.5%, respectively. Typical distribution of individual PCBs in tissues of cormorants can be seen in **Figure 66**. Similarly, in buzzards, PCB 180, 153, 187, 138 and 170 were the most abundant congeners with 27.1%, 25.7%, 8.8%, 8.6% and 8.5% contributions, respectively. No greater differences of PCB patterns were measured in herons where the same PCB 153, 180, 138 and 187 accounted for 25.1%, 17%, 11.5% and 7.3%, respectively. Relative abundance of 7 indicator PCB congeners in raptor specimens from the Záhlinice Ponds generally corresponding with the other samples is shown in **Figure 59** (together with PCBs in fish prey of aquatic birds of prey). The dominance of hexa- and hepta-CBs is obvious in all three species.

The median levels of DDTs ranged from 7.42 μg/kg ww in buzzards (2.1-470.9 μg/kg ww) to 207.5 μg/kg ww in grey herons (with a range of 2.1-2952.3 μg/kg ww). The median concentration of ΣDDTs determined in cormorants was 43.6 μg/kg ww (ranging from 0.9 to 1885.7 μg/kg ww). *p,p'*-DDE was the most abundant compound comprising 74.4% of total DDTs in buzzards, 91.5% in grey herons and 92.9% in cormorants. Surprisingly, the second most abundant DDT compound was the parental *p,p'*-DDT isomer, accounting for on average 7.2% DDTs, while the aerobic metabolite *p,p'*-DDD had only tiny share in total DDTs (on average 4% in all species). When we look at **Figure 60**, we can see and compare relative abundances of DDT compounds in fishes and birds of prey from the Záhlinice area. However, the ratio of DDTs in buzzards here is a bit different to that average including specimens from the other localities. Moreover, the concentrations of both DDTs and PCBs in the buzzards from Záhlinice were rather low (median value of ΣDDTs was 5.2 μg/kg ww) compared to the other sampling localities (median value of 24.3 μg/kg ww). Together with the fact that all the specimens from Záhlinice were youngsters (<1 year old) with no fully developed metabolism and that buzzards are feeding in terrestrial environment, there can be various interspecies and intraspecies patterns of DDTs.

As for the concentrations of HCB and HCHs in the raptors investigated, median levels were several times lower than those found for the former two groups of organochlorines. Shown in **Fig. 62**, the concentrations of HCB were mainly in units of μg/kg ww, contrary to HCHs the values of which present in the sample approx. one order of magnitude lower. The highest levels of HCB were found in herons ranging from 0.18 to 53.17 μg/kg ww (median value was 9.1 μg/kg ww), then in cormorants with 0.2-26.6 μg/kg ww (4.19 μg/kg ww) and in buzzards with a range of 0.02-7.33 (0.68) μg/kg ww. As for HCH isomers, it was hard to decide which isomer was predominant due to their very low concentrations. Despite this, β-HCH was the most abundant isomer accounting for about 77% of total ΣHCHs in buzzards, 56% in herons and 47% in cormorants. Similarly, Covaci et al. reported the predominance of β-HCH in cormorants from the Danube Delta [**127**]. However, the contribution of γ-HCH in eight cormorants from the Záhlinice Ponds (2007) was the highest (on average 51%) among HCHs. The levels of HCHs and HCB in livers of raptors were generally equal or slightly lower than in muscle. Owing to the age of the specimens investigated (youngsters or just above 1 year), this could be caused by relatively rapid growth and different metabolic activity than in adults. Moreover, no correlation was observed between the levels of HCB and HCHs

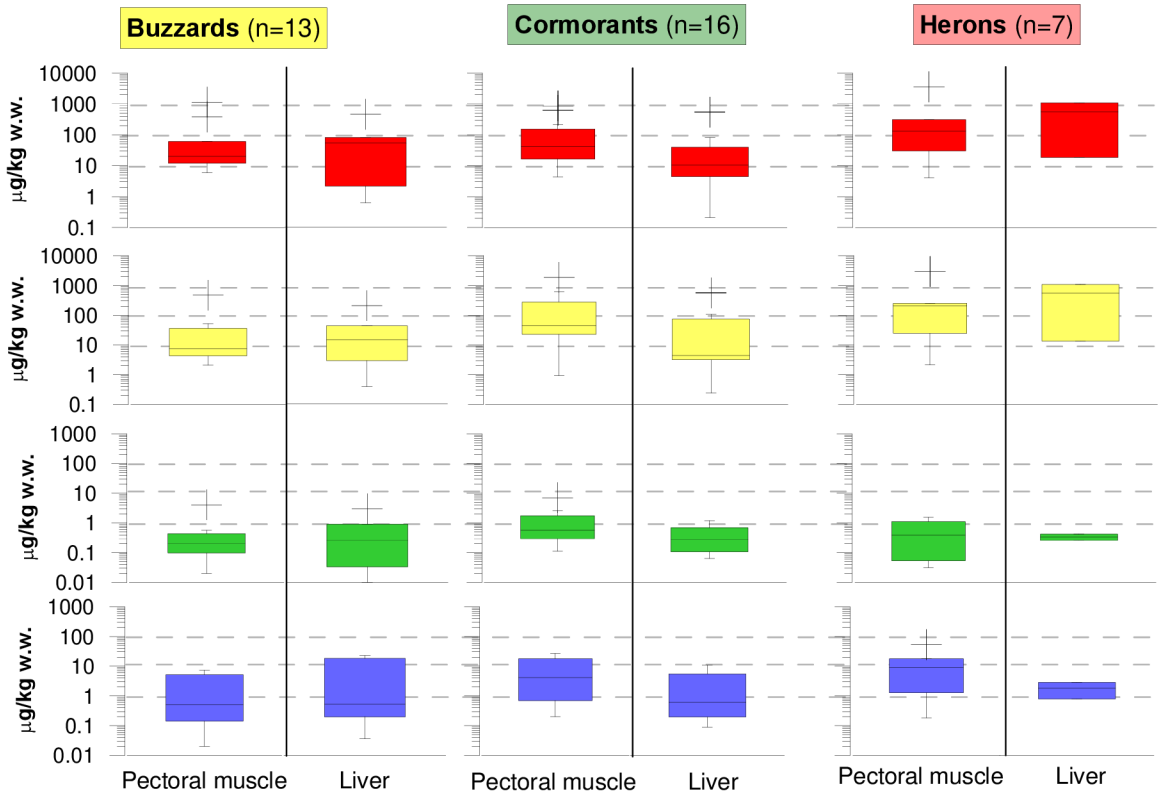


Fig. 62: Medians, 1st and 3rd quartiles, and min/max values of **Σ22PCBs**, **ΣDDTs**, **ΣHCHs** and **HCB** in pectoral muscle and liver tissues of three raptor species – summary graph. Outliers marked as (+).

in muscle and liver ($p < 0.01$). However, as can be seen in **Fig. 63**, the concentrations of *p,p'*-DDE and ΣPCBs (ln-tranformed) were highly correlated for the total data ($n = 35$, $r = 0.882$, $p < 0.01$). Van Drooge et al. [**245**], who studied organochlorine residue levels in livers of 18 raptor species from Spain (1990-1998), reported similar correlation for their data ($n = 130$, $r = 0.728$).

The other chlorinated pollutants monitored accounted for a small minority of total OCs. Cis- and trans-chlordane, oxy-chlordane, methoxychlor and mirex were the most abundant contaminants. Since there was no or only limited use of these pesticides, their levels in raptors were by far lower than those of PCBs or DDTs. For instance, in herons, mirex was determined in range of nd (not detected)-8.9 µg/kg ww, nd-7.1 µg/kg ww in buzzards and nd-8.8 µg/kg ww in buzzards. Oxy-chlordane ranged from nd to 3.8 µg/kg ww in cormorants, from nd to 2.8 µg/kg ww in herons and from nd to 11.6 µg/kg ww in buzzards. An overview of the values of all monitored organochlorines in tissues of cormorants from Záhlinice is presented in **Annexe IV.**

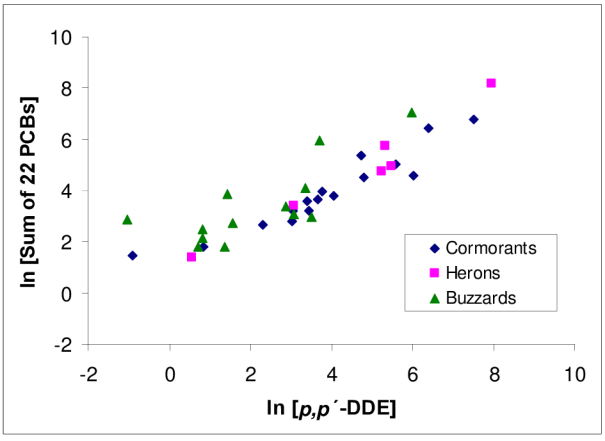


Fig. 63: Relationship between the ln-transformed concentrations (ng/g ww) of *p,p'*-DDE and ΣPCBs in 3 species of raptors from the Czech Republic ($n = 35$, $r = 0.882$)

Recent levels of organochlorine pollutants in birds of prey from different countries are presented in **Table 22**. In general, the levels of POPs found in specimens from Moravia do not significantly differ from those determined in other raptors from foreign countries. When the values expressed on lipid weight basis are compared, we can see that PCBs (22 congeners) in cormorants from the Záhlince Ponds ranged from 790 to 25,686 ng/g lw (median value was 4341.5 ng/g lw), and they were similar to the findings from Switzerland, but slightly higher than those from Romania (the median level of ΣPCBs in cormorants from this study was 41.8 μg/kg ww, ranging from 4.4 to 867.1 μg/kg ww). Herons with PCBs in a range of 883.8 to an extreme of 169,565 ng/g lw (median value of about 5,000 ng/g lw) were relatively comparable cormorants and sparrow hawks from Switzerland. But we must bear in mind that the concentrations of POPs do not follow normal distribution and large variances among specimens even from one locality (contrary to fish where such differences are not usually observed) make every complex comparison considerably difficult. However, levels of PCBs and DDT are expected to keep decreasing (though slowly) owing to restrictions and legislation measures.

Tab. 22: Recent levels of organochlorines in raptor species from different countries (ng/g ww)

Species	**Country** (sampl. year)	**ΣPCBs**	**ΣDDTs**	**ΣHCHs**	**HCB**	**Ref.**
Kestrel [b]	Italy (1991)	2722-11319	836-8685	<40 [c]	16-60.4	**[244]**
Golden eagle [b] Merlin [b] Sparrow hawk [b]	Norway (1991-1997)	264-4715 1396-1968 4489-5701	- - -	- - -	- - -	**[241]**
Whitebacked vulture	South Africa (1993-95)	-	1.65-6.9	113.1-367.4	-	**[243]**
Cormorant [b]	Greece (1997)	3.4-172.5	-	5.66-566.2	-	**[128]**
Cormorant *(Phaloc. carbo)*	Romania (2001)	26.4-69.1	74.9-137.9	6.9-9.9	2.5-4.8	**[127]**
Kestrel [b] Black kite [b]	Czech Republic (2001)	37-44.3 28.3-40.5	- -	- -	- -	**[242]**
Neotropic cormorant [a] Great grebe [a]	Argentina (2002)	- -	1612.6 2570.1	642.1 197.99	- -	**[240]**
Common buzzard [a] Sparrow hawk [a] Cormorant [a]	Switzerland (2003-2005)	173-5351 1789-79836 4186-17921	- - -	- - -	- - -	**[122]**

[a] OC in ng/g lw; [b] measured in eggs, values in ng/g lw; [c] γ-HCH;

Tissue-specific distribution of organochlorines in cormorants

Common determination of organochlorines in wildlife (fish, game birds and mamals) is mostly concentrated on muscle and liver since these are usually consumed edible tissues. But analyses of other tissues and organs or complex evaluation of the distribution of pollutants among various tissues are of rare concern. For instance, only few data exist on the distribution of PCBs to brain of raptors [**122**] and papers dealing with the use of bird feathers and hair for environmental monitoring are less common as well, contrary to eggs. This study was therefore carried out to contribute to this issue and to compare the results with those published in recent papers.

In total eight specimens of common cormorant was caught at the Záhlinice Ponds locality and their tissues were investigated for the levels of POPs, as described in more detail in **Chapter 4.1.3**. Shown in **Figures 64** and **65** are the values of PCBs, DDTs, HCHs and HCB determined in nine different tissues and organs of cormorants. Prior to any evaluation of the data, we should take the age of the investigate specimens into consideration (<1 year) since

the metabolism and feeding differ from adult specimens (growth rate, size of prey, etc). Moreover, we assessed the differences in concentrations and patterns of POPs among different tissues rather than absolute values since this was already discussed in previous chapter.

The levels of PCBs in lipids of individual tissues showed relatively strong correlations (from $r = 0.601$ for femoral muscle-kidney to $r = 0.968$ for femoral muscle-pectoral muscle) and a simple ANOVA test did not prove significant differences in the whole set of data ($p < 0.01$). However, as can be seen in **Fig. 64**, the levels of PCBs as well as DDTs, HCHs, and HCB in the lipids of the brain were obviously lower than in the other tissues. This was also observed when lipid-normalised concentrations between brain and other tissues were compared (**Fig. 6**). Here, relatively smaller difference was caused by higher lipid contents in the brain (on average 6.7±1.5%) compared to e.g. femoral muscle (on average 1.9±1.5%). Naert et al. [**122**] studied the distribution of PCBs and PBDEs to brain as well and reported comparable lipid content (7.6±2.3%) and corresponding results as for lower concentrations of PCBs in the brain than in adipose tissue. They suggested that the heterogeneous distribution between brain-adipose tissues is related to some blood-brain barrier protecting the brain against the accumulation of POPs. Alternatively, different proportions of neutral lipids in the brain (predominance of cholesterol and phospholipids) and in adipose tissue (triglycerides accounted for >90% of the total lipid content) may be the cause for this variance [**266**].

Relative abundances of PCB congeners in the tissues of cormorants are shown in **Fig. 66**. Contrary to relatively uniform levels of PCBs in different tissues (except brain), individual PCBs showed varying proportion. Whereas low chlorinated PCB congeners were more abundant in intestinal contents or feathers, more persistent lipophilic congeners with higher chlorination (PCB 138, 153, 180) predominated in heart or muscles. Higher abundances of low chlorinated PCBs in intestinal contents can be ascribed to cormorants' feeding in aquatic environment where more water soluble PCBs are more abundant (see **Chapter 5.3** or **5.1.1** for comparison). On the other hand, more lipophilic PCBs tend to accumulate in fatty tissues and are metabolised more slowly. Higher proportion of low chlorinated PCBs in feathers may come from external contamination (e.g. from water), as discussed by Jaspers et al. [**267**]. They concluded that external contamination seemed of little importance for the levels of POPs in bird feathers, however, they studied common buzzard feeding in different environment than aquatic cormorants. Another explanation could be the presence of non-persistent PCB in the blood resulting in their higher abundance in feather than in muscles or liver. Interspecies differences cannot be excluded as well. Our data showed strong correlation between the values of PCBs in lipids from feathers and muscle ($r = 0.849$), but somewhat poor for DDTs ($r = 0.305$) and HCHs ($r = 0.363$) and the levels of HCHs in lipids from feathers differed significantly ($p < 0.01$) from the other tissues. Lipid-normalised HCH concentrations were significantly greater ($p < 0.01$) than those in muscle tissues as well which could suggest a different pathway of HCH distribution. Strong correlation between PCBs in feathers and muscle ($r = 0.9$, $p < 0.01$), but weaker for liver ($r = 0.57$, $p < 0.05$), was published by Jaspers et al. [**268**]. Similarly to our findings, Jaspers et al. reported slightly higher contribution of low chlorinated PCBs in feathers than in liver tissue [**267**].

Shown in **Figures 67** and **68** are relative abundances of DDTs and HCHs in the analysed tissues of cormorants, showing no differences in distribution contrary to PCBs. The only higher contribution of *p,p'*-DDD in the intestinal contents probably come from fish prey having higher ratio of DDD/DDE (**Fig. 60**).

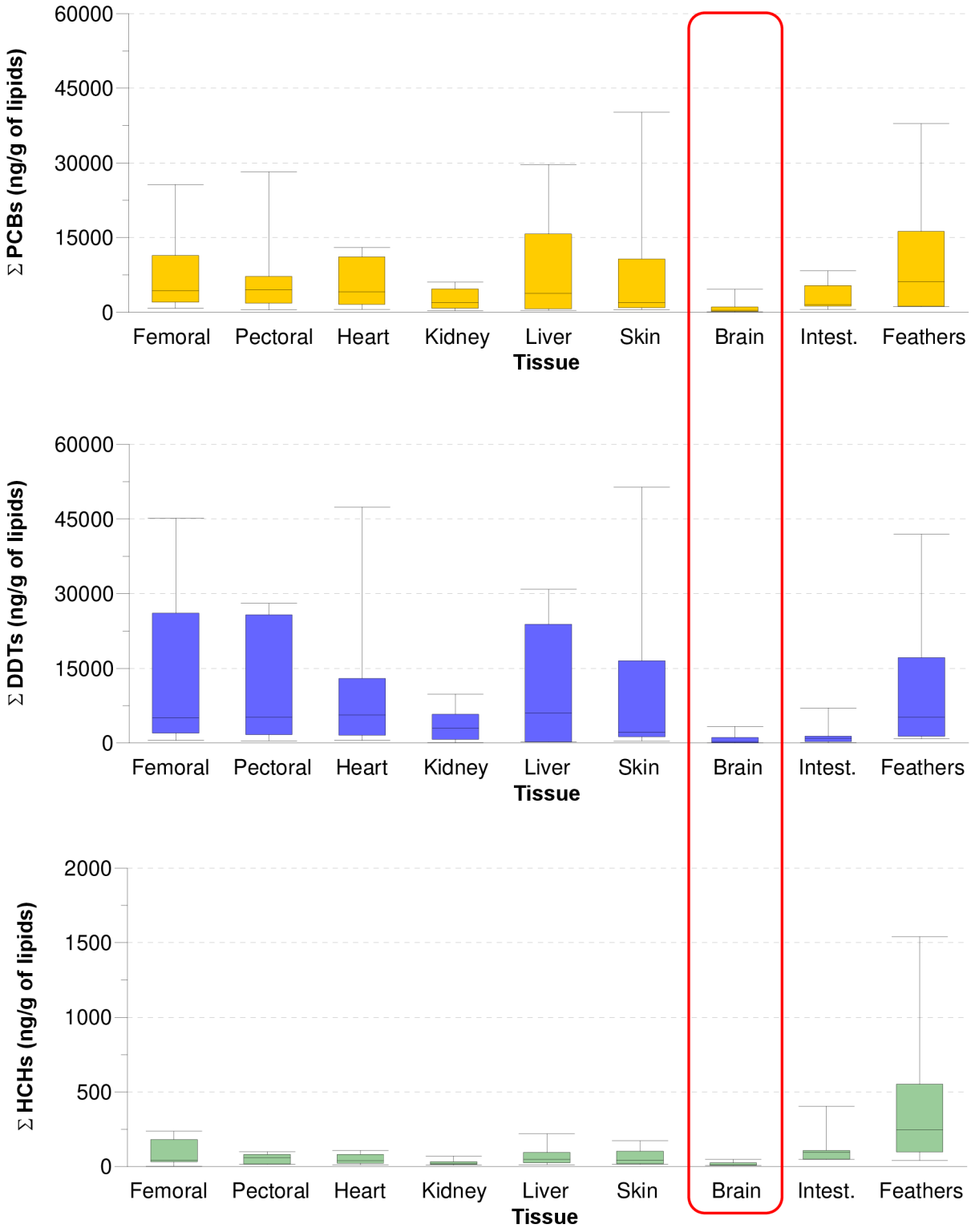


Fig. 64: PCBs, DDTs and ΣHCHs in tissues of eight cormorants from the Záhlice Ponds (values expressed as ng/g lipid weight). Box-whisker plot show median, the 1st and 3rd quartiles, min and max values.

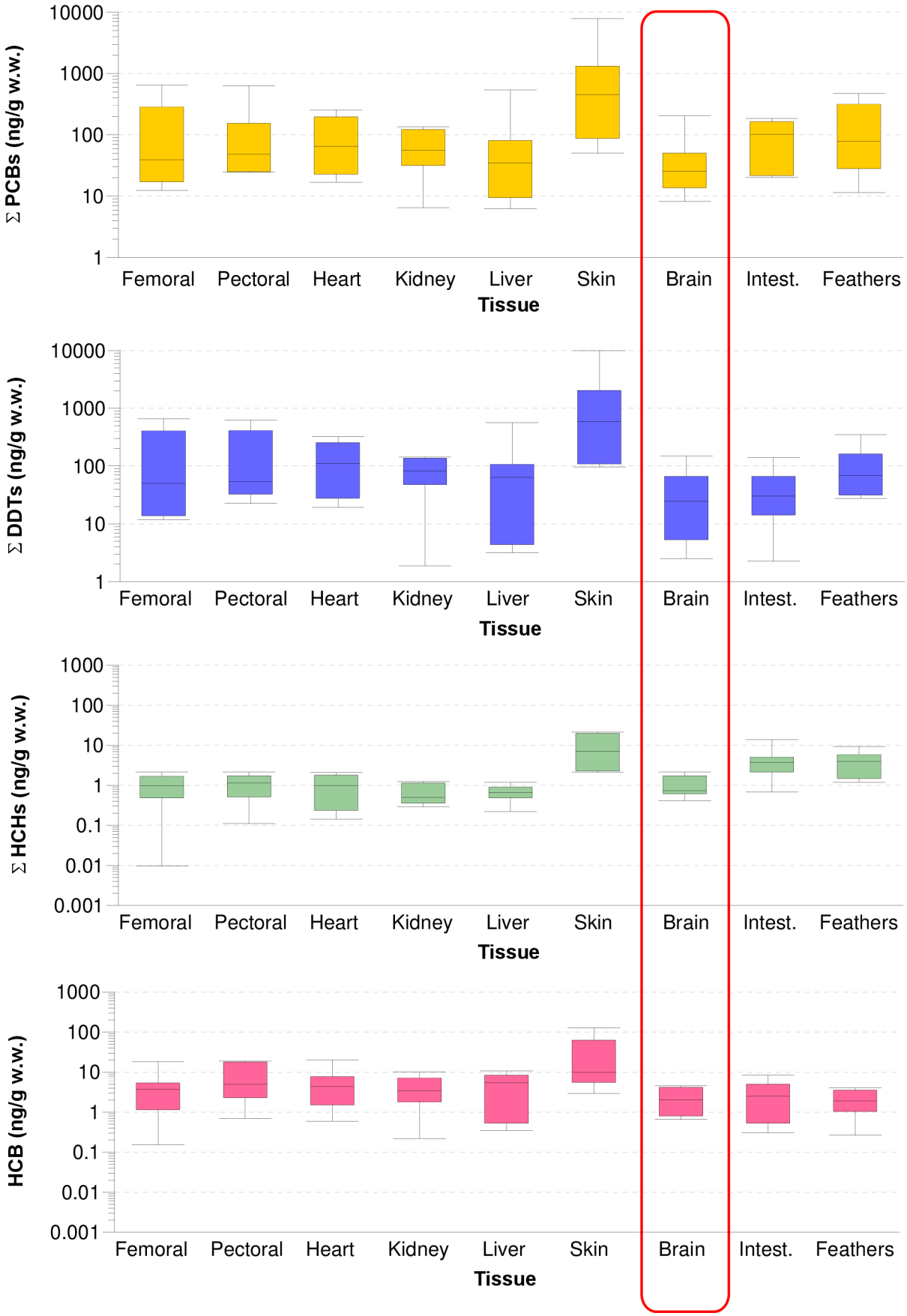


Fig. 65: PCBs, DDTs, HCHs and HCB in tissues of eight cormorants from the Záhlice Ponds (values expressed as ng/g wet weight). Box-whisker plot show median, the 1st and 3rd quartiles, min and max values.

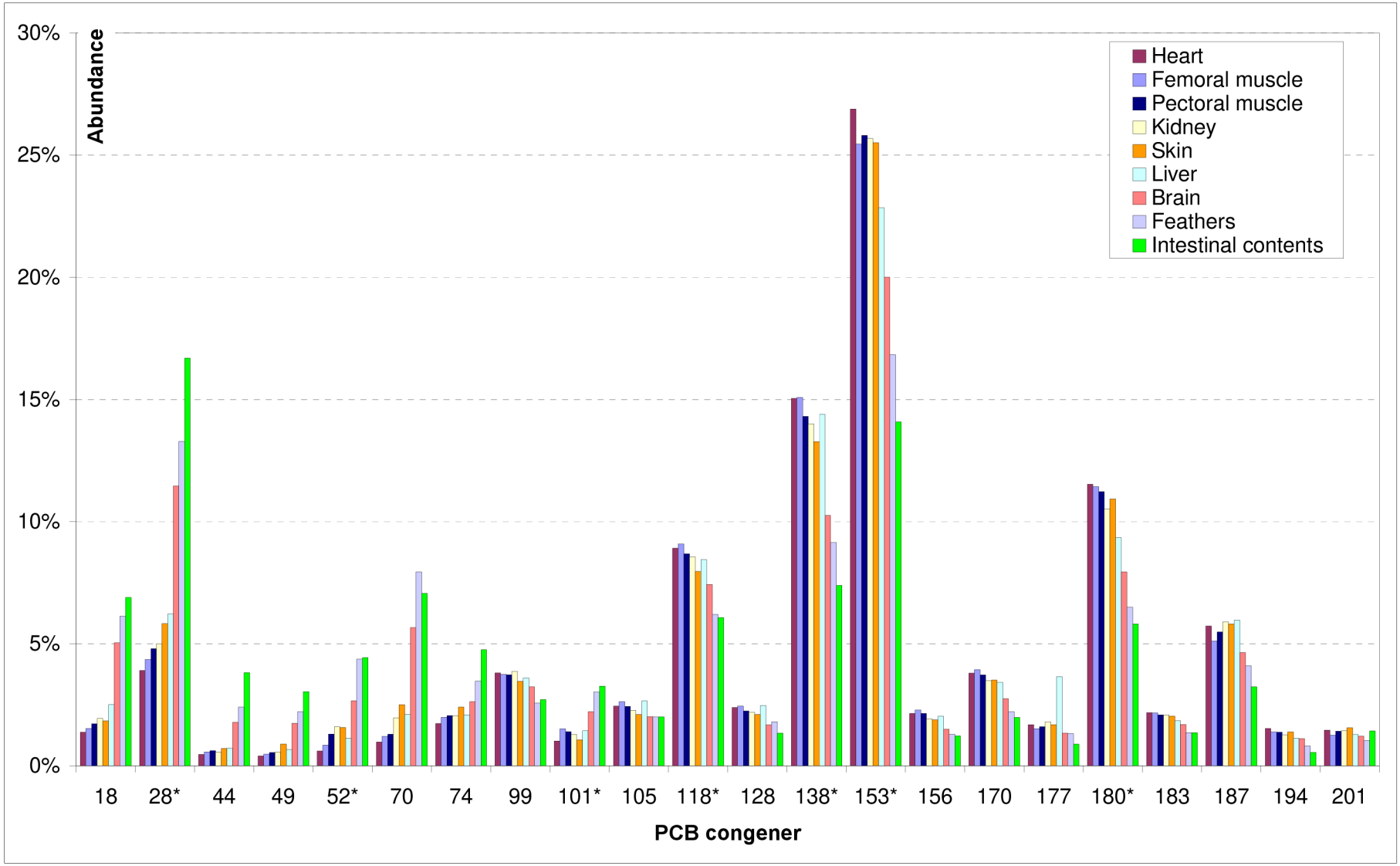


Fig. 66: Relative abundances of PCB congeners in different tissues of cormorants from the Záhlinice Ponds.

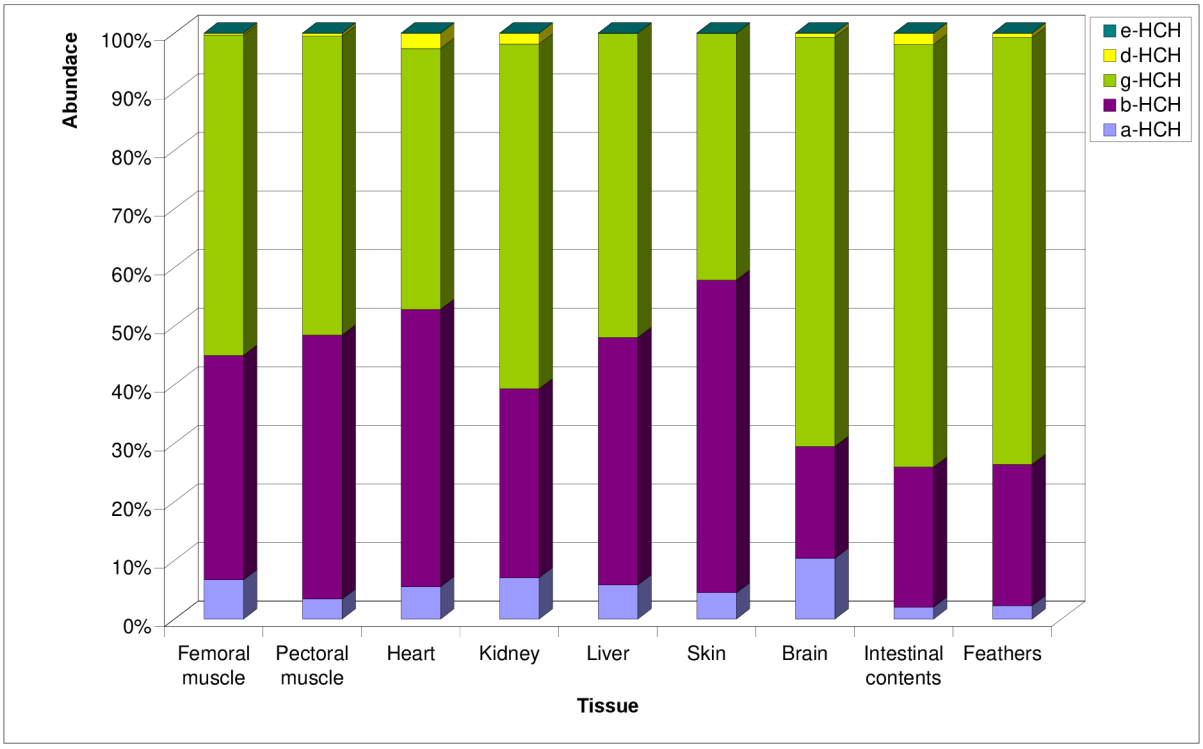


Fig. 67: Relative abundances of HCH isomers in different tissues of cormorants from the Záhlinice Ponds.

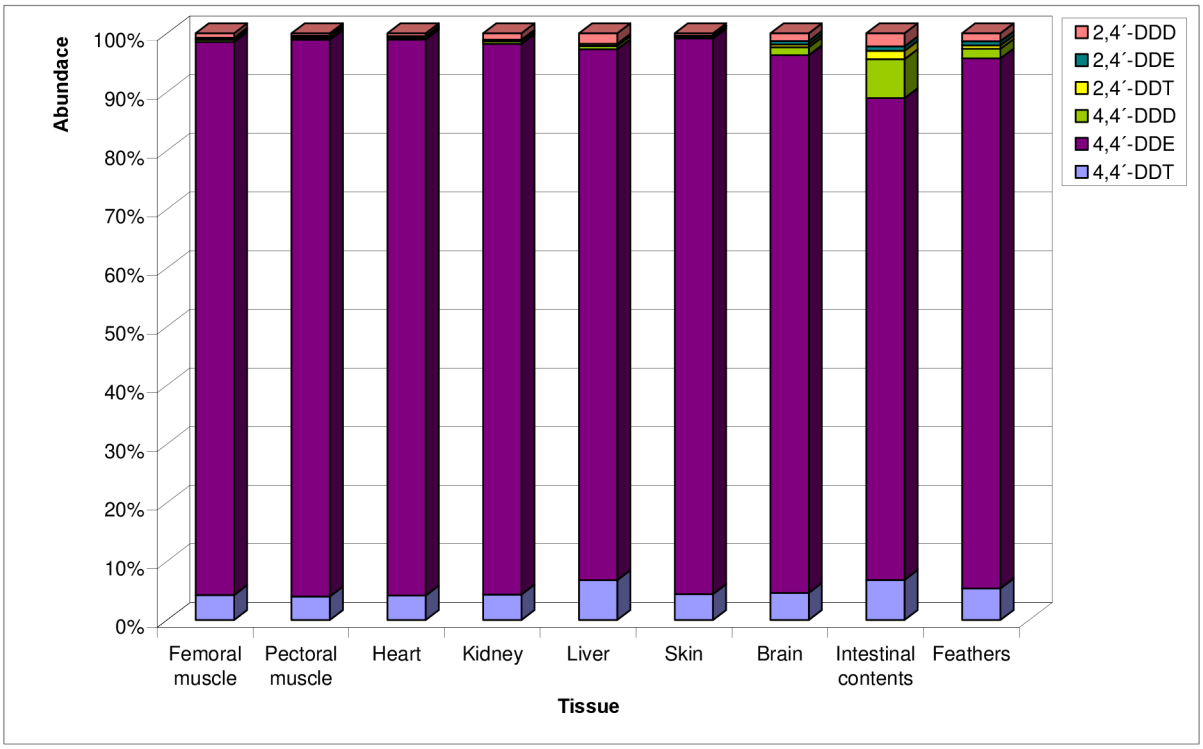


Fig. 68: Relative abundances of DDT metabolites in different tissues of cormorants from the Záhlinice Ponds.

To draw conclusions from the results of this chapter, we would say that birds of prey proved to be a sensitive tool for environmental monitoring as for the contamination with persistent organic pollutants. Together with the analyses of fish prey, the process of bioaccumulation of POPs in an aquatic food chain from the Záhlinice Ponds was demonstrated and the patterns of individual organochlorine compounds and their distribution in the food web members were described. The levels of POPs determined in three raptor species from Southern and Central Moravia did not significantly differ either mutually and when compared to those of foreign origin. PCBs and DDTs were the most abundant contaminants in the raptors' tissues, whereas HCB, HCHs and other chlorinated pesticides were determined in much lower concentrations.

Persistent pollutants showed different distribution in tissues of cormorants where the brain was the least loaded tissue, compared to the other organs with rather equal levels of POPs. Moreover, different abundances of individual PCB congeners in the tissues suggested different pathways of their transport and accumulation to the organs. And finally, bird feathers appeared to be a suitable non-destructive biomonitoring tool as the levels of main organochlorine pollutants incl. PCBs and DDTs were quite comparable between feathers and commonly analysed muscle or liver.

5.4. Comparison of microwave-assisted extraction and pressurised solvent extraction for the determination of organochlorines in various matrices

The extraction of analytes from the sample matrix is regarded as a critical step in the whole analytical process. Besides classical methods as Soxhlet or ultrasonication extraction used for extraction of POPs from both abiotic and biotic samples, new improved techniques have been developed to reduce the extraction time and solvent volume, and to increase the recovery of analytes. Thus, the aim of this study was to compare the accuracy and extraction efficiency of two modern techniques that are available in the analytical laboratory of the ICTEP and that are commonly used for determination of POPs in environmental samples. The extraction parameters have been chosen from several important papers dealing with this topic.

In order to facilitate an appropriate comparison of the extraction efficiency of the techniques used, and to compare our results with those from other studies, solvent mixtures as well as the method parameters reported to be sufficient enough for the extraction of chlorinated POPs were chosen. The blend of *n*-hexane:acetone (1:1, v/v) has been used in many similar studies (see above), the use of the mixture *n*-hexane:acetone (7:3, v/v), on the other hand, was recommended by the application note of the Anton Paar Multiwave 3000 extraction system [**226**]. A third solvent mixture, n hexane:DCM (1:1, v/v), was used, together with a *n*-hexane:acetone (4:1, v/v) by Suchan et al. [**161**] for the isolatation of chlorobiphenyls and pesticides from fish muscle tissue by means of PSE and Soxhlet extraction. The extraction conditions of both techniques tested here follow the recommendations from similar studies [**133**, **161**, **227**, **136**].

Microwave-assisted extraction

The parameters of the applied MAE method were the same for all samples and extraction mixtures used. The determined concentrations and recoveries from different extraction procedures and matrices are shown in **Figures 69 to 73**. Extraction efficiency and precision

(in %) related to the certified reference materials used are summarised in **Table 23**. Considering the recoveries and precisions of target analytes concentrations based on the reference material analyses, there were no significant differences between the two solvent mixtures used for the MAE experiments. Slightly higher concentrations of both PCBs and OCPs were determined in soil and sediment samples (**Figures 72** and **73**), including the BCR 536 ref. material, when the *n*-hexane:acetone (7:3, v/v) was used. For instance, Enders and Schwedt found the highest recoveries for the extraction of PCBs from soil and sewage sludge by using *n*-hexane:acetone (3:1) [**228**]. Contrary to the PSE experiments, *n*-hexane:DCM (1:1) was not used for the MAE experiments, since this mixture does not absorb microwaves and hence the use of heating elements would be necessary. In addition to that, results obtained from PSE showed no significant differences between *n*-hexane:acetone (1:1) and this mixture. Noticeably outlying values of recoveries found for PCB congeners 105, 128, and 156 in the BCR 536 sample could be caused by co eluting peaks affecting the final concentration. However, these congeners were present in the sample on approx. one order of magnitude lower levels compared with the other compound, as can be seen in **Figure 70**, and thus potential co-elutions might affect the found valued in large measure. The possibility of processing up to 16 samples in one run, together with fast and efficient extraction, appeared to be the main advantages of this method. On the other hand, crude extracts remained in the extraction cylinders together with the sample matrix after the extraction programme was completed, and thus either centrifugation or filtration was necessary to obtain a relatively clear extract.

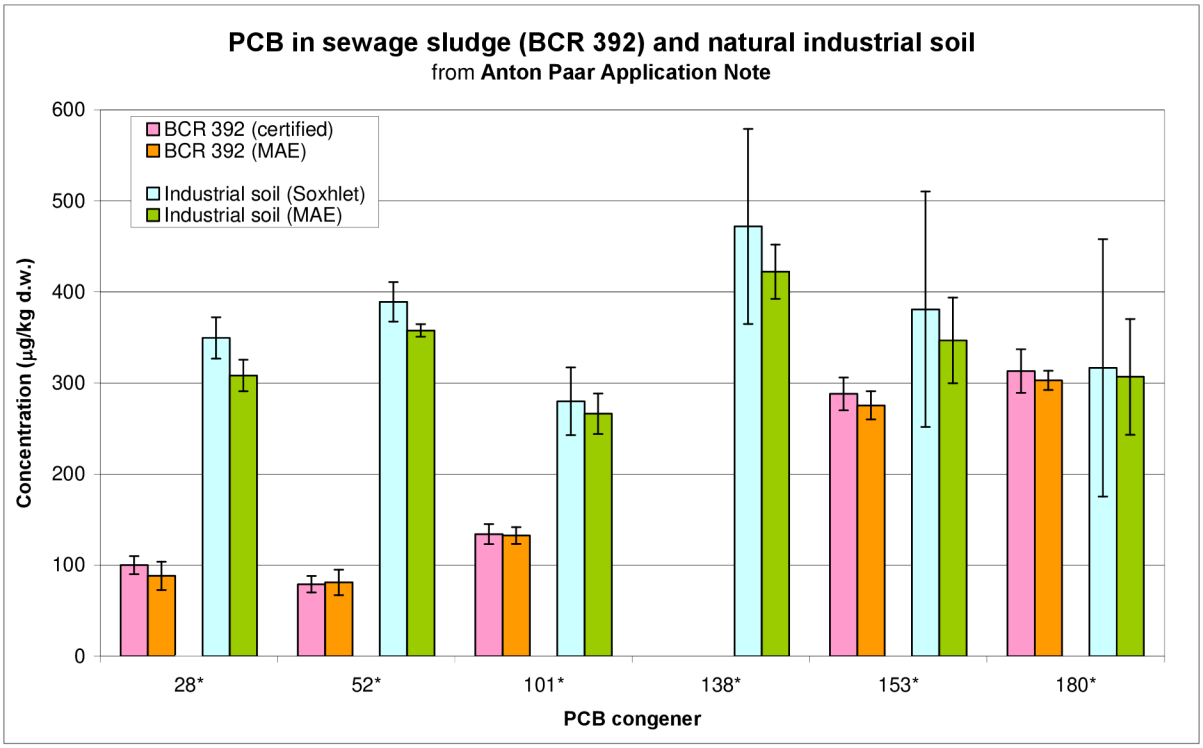


Fig. 69: Concentrations of individual PCB congeners in two certified reference materials determined by microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE). Error bars represent SD (n = 4), the uncertainty of the ref. material is expressed as combined uncertainty U_c (k=2).

Pressurised solvent extraction

PSE, as well as MAE, employs elevated pressure and temperature to facilitate the penetration of an extraction solvent into the sample matrix, leading to higher recoveries of analytes and shorter extraction times, in comparison to classical extraction methods. Concentrations of PCBs and OCPs determined in the samples analysed by PSE are presented in **Figures 70** to **73**, while the calculated recoveries based on the certified values from the reference materials are summarised in **Table 23**. Similarly to the results from the MAE trials, no significant differences were found in the concentrations determined by different extraction solvents. The same conclusion can be drawn when the results from both extraction methods were compared. Some PCBs congeners and pesticides showed a somewhat higher recoveries for the *n*-hexane:acetone (7:3, v/v, PSE B) mixture, but on the whole, the differences were not statistically important. The recoveries obtained using *n*-hexane:dichloromethane (1:1, PSE C) were, except for individual deviations, quite comparable to those from the *n*-hexane:acetone (1:1, PSE A) blend. Sporring et al. [**133**] and Suchan et al. [**161**] found out that the number of extraction cycles have an influence on the recoveries and recommended two static cycles as a suitable number for good results. Excellent extraction efficiencies of 96-99% for PCBs in sediment using a *n*-hexane:acetone (1:1) mixture and a 2 × 5 min extraction were found by Josefsson et al. [**229**]. In comparison to those from MAE, relatively pure extracts were obtained using a specific approach described in [**230**, **173**]. The extraction cell was half filled with Florisil® powder in order to retain co-extracted compounds and on this layer the sample mixed with anhydrous Na_2SO_4 was added. The clean-up was effectively combined with the extraction step and the extracts obtained this way were clear enough to be analysed directly by a GC/ECD. However, it was not possible to use this approach for lipid-rich fish tissue samples, since the volume of the extraction cell used was too small to be filled with the required amount of the sorbent. Therefore, an additional clean-up on an adsorption column was performed to remove lipids.

Tab. 23: Extraction efficiency and precision of microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE) related to the values of the used certified reference materials

PCB No.	BCR® 536 (Chlorobiphenyls in freshwater harbour sediment)					BCR® 718 (PCBs in canned fresh herring)		
	MAE A	**MAE B**	**PSE A**	**PSE B**	**PSE C**	**MAE A**	**MAE B**	**PSE A**
	Extraction efficiency + RSD (%) for n = 4					Extraction efficiency + RSD (%) for n = 4		
28	88.4 ± 9.2	92.4 ± 6.8	107.3 ± 6.8	110.2 ± 4.1	106.1 ± 5.5	76.8 ± 24.7	67.1 ± 29.6	86.0 ± 16.0
52	93.2 ± 7.8	99.8 ± 5.6	114.4 ± 5.6	115.9 ± 8.5	108.9 ± 6.9	103.0 ± 7.4	96.3 ± 5.7	84.5 ± 9.5
101	77.8 ± 10.7	86.8 ± 8.7	81.2 ± 11.6	90.9 ± 9.1	86.9 ± 9.0	97.5 ± 2.5	98.2 ± 4.7	86.6 ± 8.0
105	140.7 ± 14.4	145.7 ± 24.1	177.2 ± 20.8	207.9 ± 20.0	182.1 ± 22.9	107.5 ± 5.4	88.9 ± 6.6	116.7 ± 12.3
118	95.5 ± 9.7	108.5 ± 5.8	122.0 ± 7.7	122.9 ± 6.0	111.4 ± 6.0	96.6 ± 7.6	94.9 ± 7.1	95.5 ± 11.3
128	156.5 ± 20.5	158.3 ± 10.9	184.3 ± 6.9	202.8 ± 18.8	189.8 ± 9.1	129.4 ± 12.5	81.9 ± 13.6	108.9 ± 5.7
138	105.7 ± 5.1	102.6 ± 5.9	113.1 ± 5.6	113.2 ± 6.0	116.3 ± 5.0	102.4 ± 2.6	93.6 ± 4.6	100.4 ± 2.8
153	97.0 ± 8.6	99.6 ± 3.5	101.8 ± 5.4	105.4 ± 5.3	103.8 ± 3.4	108.5 ± 3.7	104.2 ± 1.3	104.5 ± 2.4
156	154.2 ± 22.0	189.2 ± 20.3	196.7 ± 22.0	188.3 ± 13.8	175.8 ± 18.9	131.6 ± 26.1	139.5 ± 32.3	118.3 ± 18.5
170	91.4 ± 9.9	83.0 ± 10.7	92.7 ± 13.2	109.3 ± 13.3	96.3 ± 6.2	125.7 ± 9.9	92.7 ± 26.1	107.9 ± 8.0
180	125.2 ± 4.7	123.5 ± 10.6	138.7 ± 7.9	118.2 ± 10.9	109.5 ± 11.0	111.6 ± 10.6	108.5 ± 6.1	107.9 ± 4.2

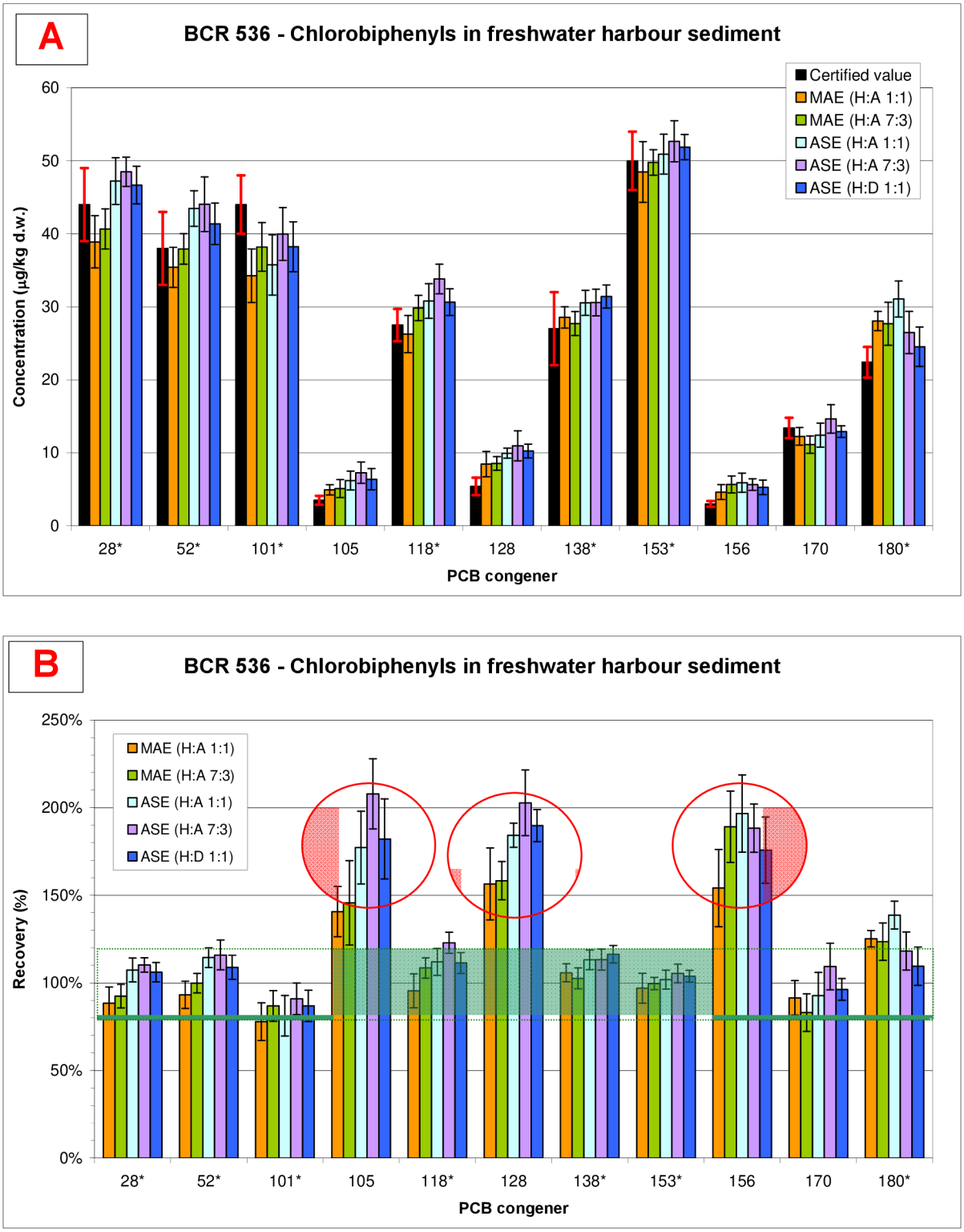
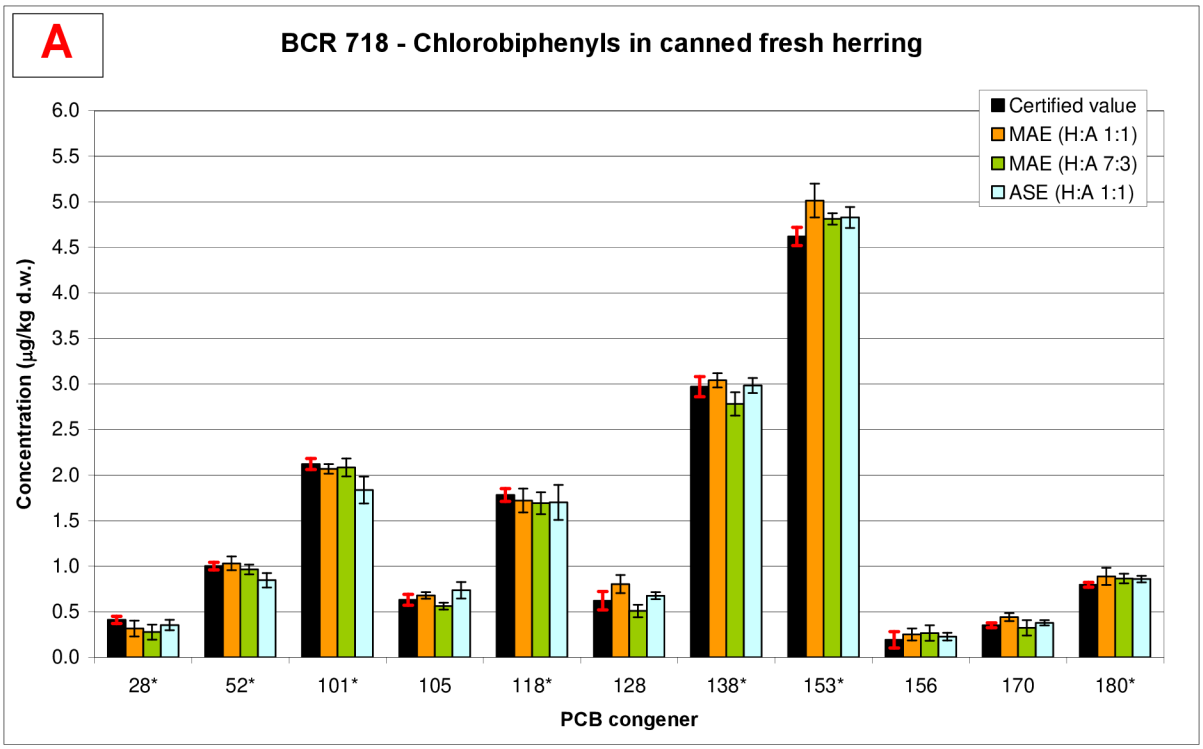


Fig. 70: Concentrations (A) and recoveries (B) of individual PCB congeners in abiotic CRM determined by microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE). Error bars represent SD (n = 4), the uncertainty of the reference material is expressed as combined uncertainty U_c (k=2).



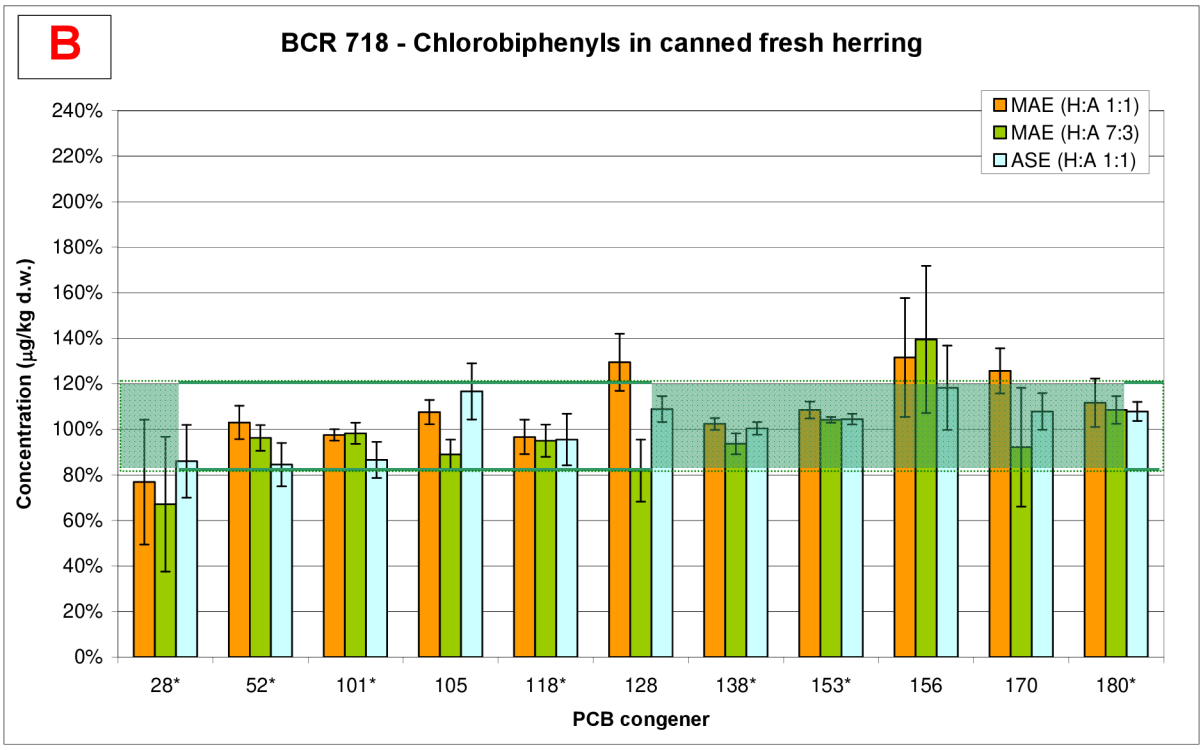
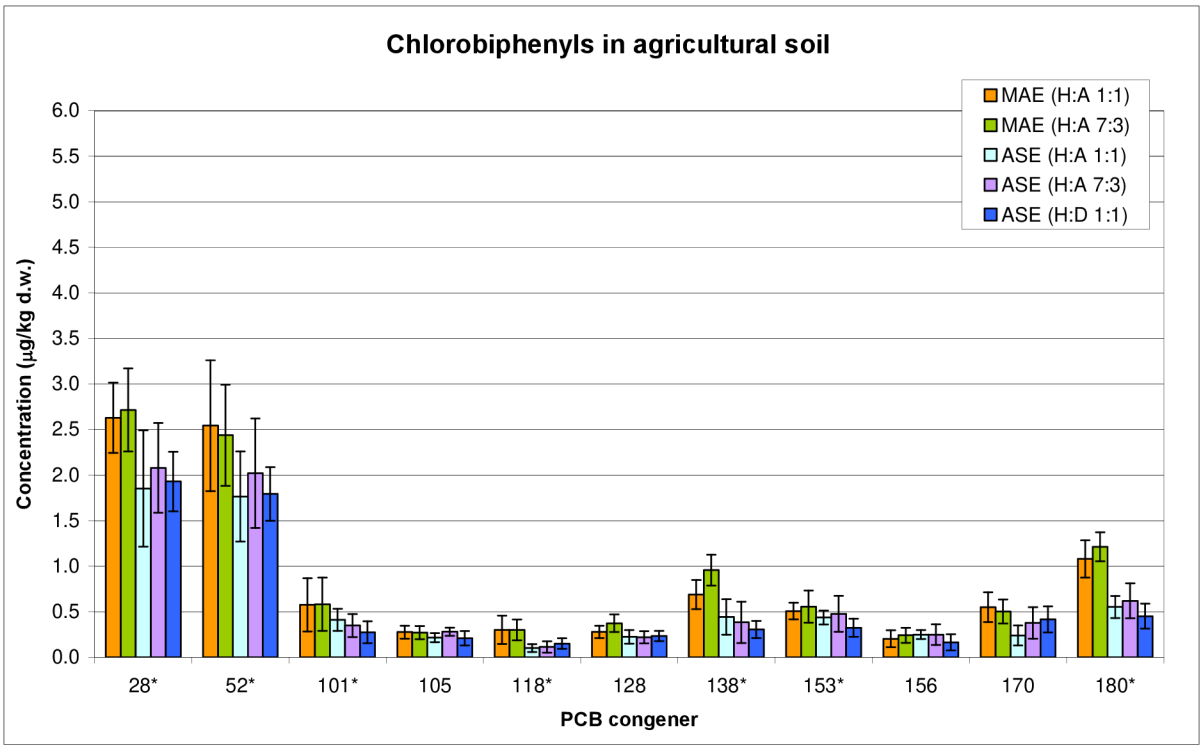


Fig. 71: Concentrations (A) and recoveries (B) of individual PCB congeners in biotic CRM determined by microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE). Error bars represent SD (n = 4), the uncertainty of the reference material is expressed as combined uncertainty U_c (k=2).



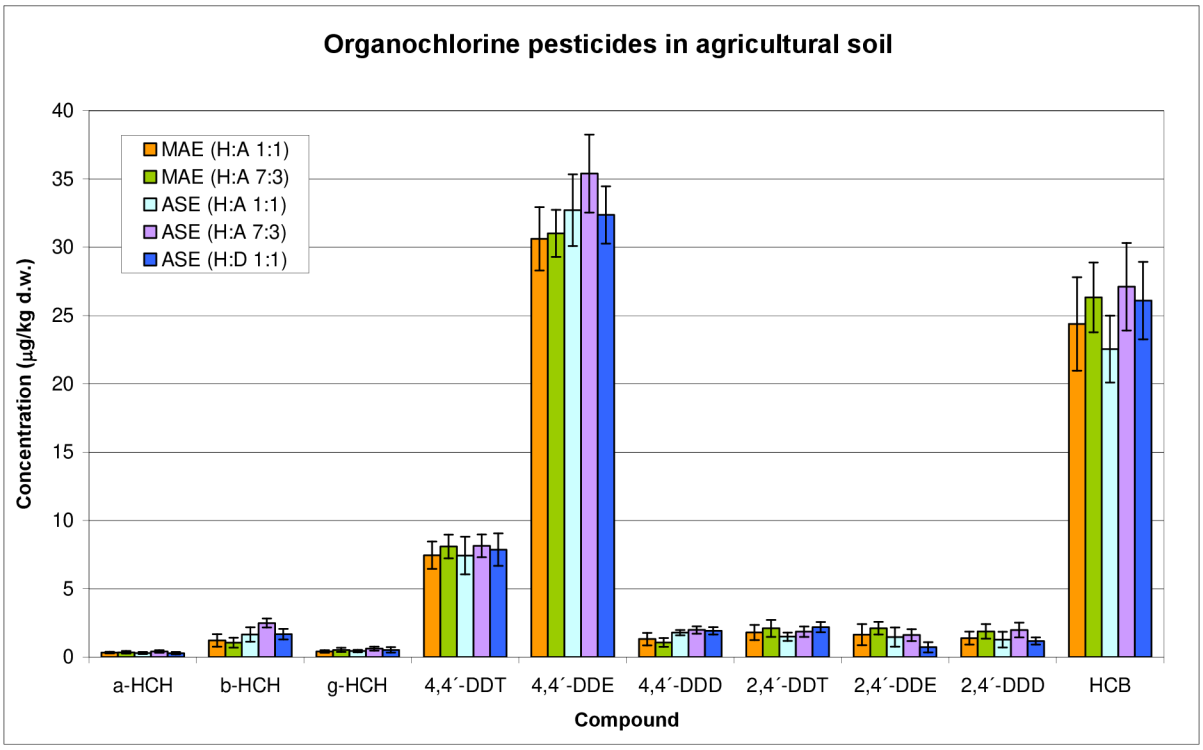
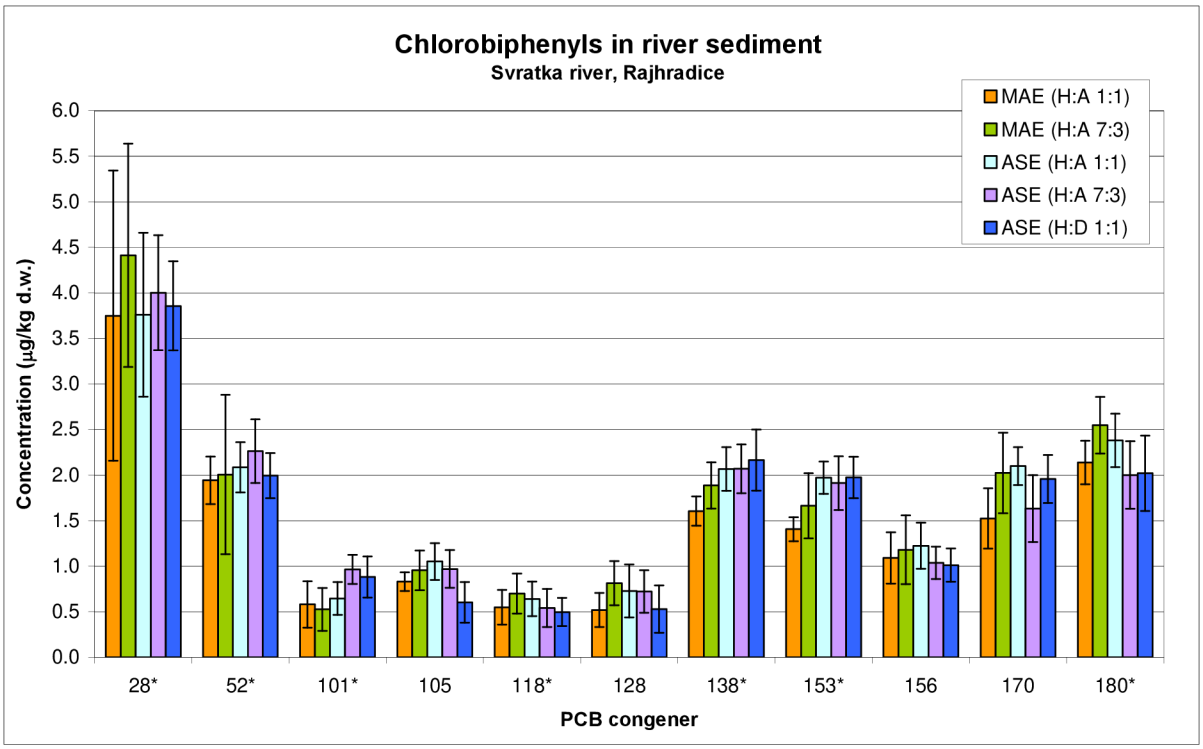


Fig. 72: Concentrations of PCB congeners and OCPs in the agricultural soil sample found by microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE). Error bars represent SD (n = 8).



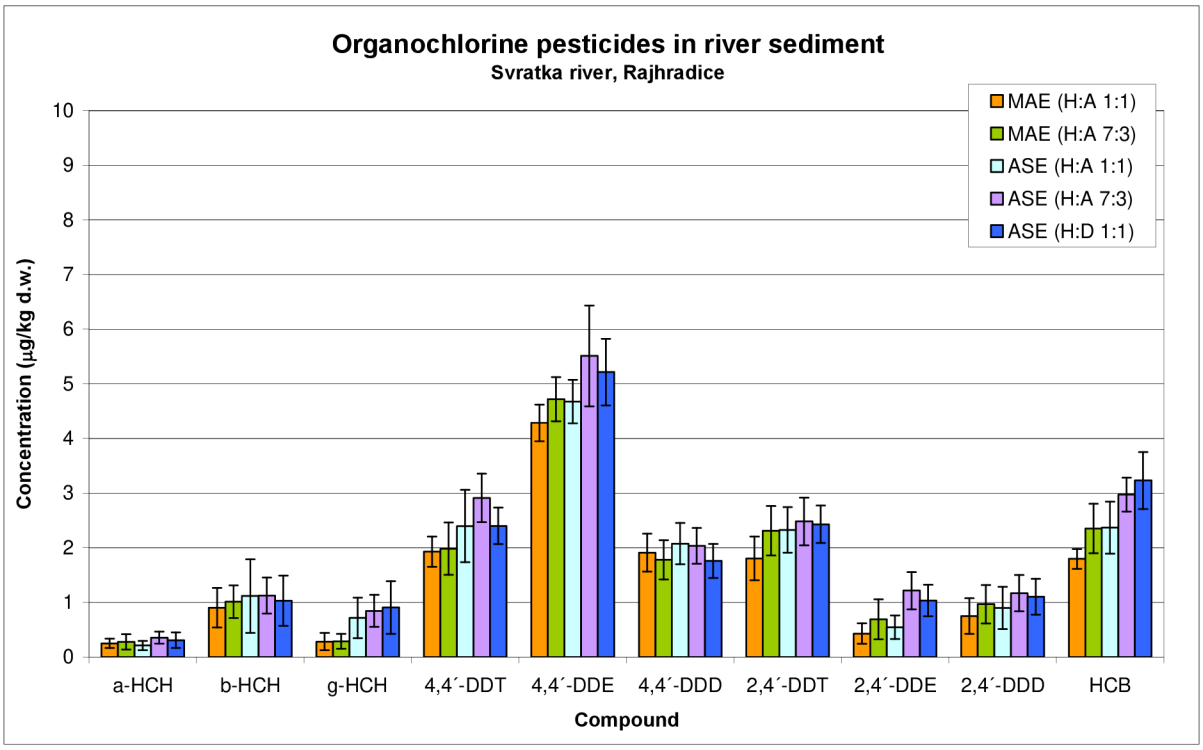


Fig. 73: Fig. 3. Concentrations of PCBs congeners and OCPs in the river sediment sample found by microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE). Error bars represent SD (n = 8).

6. CONCLUSIONS

This doctoral thesis was focused on the issue of persistent organic pollutants and their occurrence and fate in the environment in the South Moravia Region, Czech Republic. Both terrestrial and aquatic ecosystems were the environments of concern and polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were the monitored contaminants. High resolution gas chromatography with electron capture detection (HRGC/ECD) was employed for the determination of contaminants in the samples.

- The first chapter presented the results of the studies focused on the determination of POPs in aquatic ecosystems. Brno Lake, the Svratka River and several water reservoirs from Southern and Central Moravia were the localities of interest. Two independent studies dealing with the bioaccumulation of POPs in an aquatic food chain and with historical trends in POPs recorded in sediment layers were carried out at Brno Lake. Despite limited number of samples of fish specimens, the obtained results provided useful information on the current contamination of the Brno Lake's food chain and on the bioaccumulation and magnification of persistent organochlorine pollutants in sediment and aquatic biota. The concentration of PCBs in water from Brno Lake (0.32 μg/L) was much lower than that in sediment (about 33 μg/kg dry weight) and by far lower than those in predatory fish (about 3,700 μg/kg lipid weight in zander). Moreover, as the levels of POPs changed through the food chain, the patterns of PCB congeners and DDT isomers and metabolites showed changes as well. More soluble PCB 28 accounted for 70% of total PCBs in water, whereas its contribution in asp fish was only 1.7% contrary to higher chlorinated PCB 138, 153 and 180 with 77.2% of total PCBs. As for DDTs, the *p,p'*-DDE was the most abundant compound in all samples, followed by *p,p'*-DDD. Parental *p,p'*-DDT accounted for only several percent and suggested that there are no more important sources of contamination. HCB and HCHs were detected in much lower concentrations than PCBs and DDTs and did not show any clear accumulation within the evaluated food chain.

  Analyses of sediment cores from Brno Lake provided useful information on historical changes in the levels of POPs during recent decades. Based on the sedimentology and the total organic carbon content, an increase in concentrations of organochlorine pollutants was observed starting in about the early 1940s. The maximum values were found at a depth of approx. 70-77.5 cm (about 77 μg/kg dry weight) and from this depth up the values have declined to the current 40 μg/kg dw. Compared to other similar findings published, the occurrence of maximum values in sedimentary layers dated as back as the 1980s followed with a decrease up to now can be regarded as a general global trend reflecting the international efforts to limit the PCB and DDT use during past decades.

  Determination of POPs in chub specimens from the River Svratka aimed at assumed differences between two sampling sites, caused by the presence of wastewater treatment plant upstream from the first locality. The samplings were performed twice (in spring and autumn) and the results from the four sets of samples were evaluated. Mean levels of PCBs in muscle of chubs from Modřice were 41.9 and 30.8 μg/kg ww for the spring and autumn sampling, respectively, and at Rajhradice, the means were determined as 38.8 and 38.6 μg/kg ww, resp. Predominating PCB congeners in all tissue were the higher chlorinated

CB 138, 153, and 180. Concentrations of DDTs were similar to those of PCBs and the *p,p'*-DDE highly predominated in all tissues, followed by *p,p'*-DDD and *p,p'*-DDT the contributions of which were quite comparable. The levels of HCHs, HCB and OCS were of minor abundance. The highest levels of POPs were found in skin of fish, followed by viscera and muscle tissue. However, no significant differences were found, despite the fact the sampling localities were separated by a weir and thus fish were not supposed to migrate between them, probably due to relatively short distance between the sampling localities. The values did not exceed any hygienic limits for freshwater fish and represented usual background levels in the Czech Republic.

The last subhead presented the results of an evaluation of hygiene quality of fishes caught by recreational fishing, as far as the levels of organochlorine pollutants were concerned. Concentrations of PCBs (mean of 44.7 μg/kg ww), DDTs (mean of 85.0 μg/kg ww), HCHs (mean of 2.7 μg/kg ww) and HCB in edible parts (muscle and skin) of common carps did not exceeded the valid hygienic limits, were quite comparable with other levels found in carps from ponds in the Czech Republic and the consumption of them did not pose any health risks (considered the WHO ADI values).

- An assessment of the contamination of terrestrial environment with organochlorine pollutants was performed using conifer needles as a biomonitoring tool. Due to the worldwide distribution of coniferous trees and to a high lipid content in needles, these can serve as a natural passive 'sampler' of persistent volatile pollutants. Needles from 22 localities within the Czech Republic – both rural and urban – were sampled during 2007 and investigated for the content of common organochlorines incl. PCBs and chlorinated pesticides. The obtained data showed relatively low levels of POPs in the terrestrial environment in the CR and the contamination determined in needles was supposed to originate mainly from the long-range transport via the atmosphere. Compared with some similar studies from the CR and with findings from other countries, our data on POPs in needles from CR seemed to be of little importance and just above the limits of detection in many samples. No differences were found between the POP levels in samples from rural and urban localities and the found variances were probably due to local climate conditions.

- Since birds of prey are top predators highly positioned in the food chain, they are a sensitive indicator of environmental pollution with persistent organic pollutants. Specimens of three species of birds of prey common in the Czech Republic – the grey heron, the cormorant and the common buzzard – were collected at three localities of Central Moravia during 2003-2007 and investigated for the content of PCBs and organochlorine pesticides. In addition to that, POPs were determined in sediment and fish prey of water raptors from the Záhlice Ponds in order to document the process of bioaccumulation of persistent pollutants in the food chain. An increase in levels of PCBs and DDTs was observed from bottom sediment via fish prey to the top water predators as cormorants and herons showed clearly the contaminant accumulation. Whereas the levels of PCBs in sediment were about 24 μg/kg dry weight (ppb), by far higher concentrations, reaching even tens of thousands of ppb, were determined in adipose tissues of raptors. DDTs correlated well with the levels of PCBs, while HCB and HCHs were accumulated to a less extent. Moreover, there was different distribution of PCB congeners and DDT isomers and metabolites to individual members of the food chain showing that high

chlorinated and more persistent PCBs as 138, 153 and 180 are predominant in species situated higher in the food web, whereas low chlorinated PCBs are more abundant in water or omnivorous fish. Similarly, *p,p'*-DDE, as the aerobic metabolite of insecticide DDT, highly dominated in raptor tissues whereas the ratio of *p,p'*-DDD/*p,p'*-DDE in sediment was nearly identical. The abundance of the parental *p,p'*-DDT was relatively low in all samples suggesting that there was no recent source of contamination. Comparison of the levels of POPs in three raptor species showed that there were no greater differences in levels of organochlorines among the investigated species and PCBs and DDTs were again the most abundant contaminants. The highest concentrations of PCBs were found in herons (median values of about 5,000 ng/g lw), followed by cormorants and terrestrial buzzards (median value of about 1,300 ng/g lw). The findings were comparable with those from some foreign studies, despite the fact there were significant intraspecies differences and the concentrations of POPs did not follow normal distribution. Generally, the determined levels of the contaminants seemed not to be of greater importance (except few outlying values).

The last subhead of this chapter described the distribution of organochlorine pollutants to various tissues of cormorants and evaluated the usefulness of bird feathers as a non-destructive tool for biomonitoring. The obtained data suggested that there are different pathways of transfer and accumulation of POPs in different organs of raptors. The brain was found to be by far the least loaded organ with organochlorine contaminants, contrary to the other tissues showing relatively equal load. This was attributed, among others, to different contents and composition of lipids in the tissues. And finally, bird feathers showed to be a good tool for monitoring the contamination of (at least) cormorants with POPs as the levels determined in muscle and feathers were in good correlation.

- The last chapter dealt with a comparison of the efficiency of two automated extraction techniques – Pressurised Solvent Extraction (PSE) and Microwave-assisted Extraction (MAE) – for the determination of chlorinated POPs in various samples. The recoveries of the target analytes determined by both extraction techniques and solvent mixtures including *n*-hexane:acetone (1:1, v/v), *n*-hexane:acetone (4:1, v/v) and *n*-hexane:DCM (1:1, v/v) were very similar and there were no significant differences between the values found. Both PSE and MAE methods offered accurate date with a good precision. Moreover, these techniques were found to be quite easy to operate and low solvent consuming (about 25-30 ml per one sample extracted). Similarly, the total extraction times were by far shorter (tens of minutes) than those reported for conventional Soxhlet extraction (up to 48 hrs). Using a 16-positions carrousel for the MAE experiments allowed a further reduction of the extraction time per one sample (about 5 min. including sample preparation). On the other hand, mixing the sample matrix with a suitable sorbent in the PSE extraction cell allowed to omit the clean up step which made the whole analytical process shorter and less laborious, since most co-extracts were retained inside the cell. The presented extraction techniques were found to be capable of providing quality results as far as POPs analysis is concerned. Their main advantages – high extraction efficiencies, very short extraction times and low solvent consumption – make them strong analytical tools competing with classical methods, although their purchase costs may seem much higher than those of e.g. Soxhlet or ultrasonication extraction.

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8. LIST OF ABBREVIATIONS

Ah	Aryl hydrocarbon
ASE	Accelerated Solvent Extraction
CAS	Chemical Abstracts Service
ECD	Electron Capture Detector
EPA	Environment Protection Agency (USA)
FMAE	Focused Microwave-Assisted Extraction
GLP	Good Laboratory Practice
GPC	Gel Permeation Chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HPLC	High Performance Liquid Chromatography
HRGC	High Resolution Gas Chromatography
IARC	International Agency for Research on Cancer
IgA, IgM	A- or M-Immunoglobulin
IPCS	International Programme on Chemical Safety
LD_{50}	Lethal Dose (for 50 % of tested animals)
LLE	Liquid-Liquid Extraction
MFO	Mixed Function Oxygenase
MS	Mass Spectrometry
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NOEL	No-Observable Effect Level
OCP(s)	Organochlorine Pesticides
OCS	Octachlorstyrene
PAH(s)	Polycyclic Aromatic Hydrocarbons
PBDE(s)	Polybrominated Diphenylethers
PBTs	Persistent, Bioaccumulative and Toxic compounds
PCB(s)	Polychlorinated Biphenyls
PCDD(s)	Polychlorinated Dibenzo-*p*-dioxins
PCDF(s)	Polychlorinated Dibenzofuranes
PCQ(s)	Polychlorinated Quarterphenyls
POP(s)	Persistent Organic Pollutants
PSE	Pressurised Solvent Extraction
SCF	Scientific Committee on Food (EU)
SPMD	Semi-Permeable Membrane Device
SPME	Solid Phase Microextraction
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxine
TDI	Tolerable Daily Intake
TEF	Toxic Equivalency Factor
TEQ	TCDD Toxic Equivalency
TOC	Total Organic Carbon
TOF	Time of Flight
TWI	Tolerable Weekly Intake
WHO	World Health Organisation

9. ANNEXES

Annexe I Photodocumentation

Annexe II Sample chromatograms

Annexe III Concentrations of PCBs in tissues of eight cormorants – Záhlinice Ponds

Annexe IV Concentrations of organochlorine pesticides in tissues of eight cormorants – Záhlinice Ponds

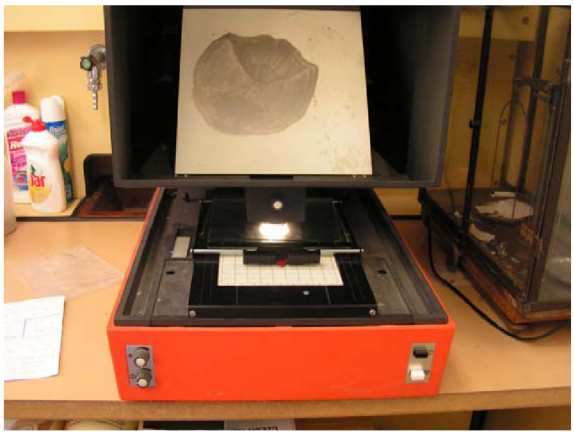
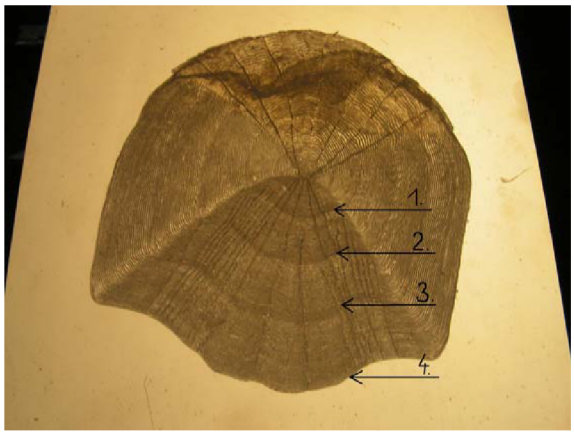
Annexe V Poster presentation of Czech Geological Survey; 4th Meeting on Chemistry Life (09/2008), Brno, Czech Republic

Annexe VI Award-winning poster presentation from EMEC8, December 2007, Scotland

Annexe VII Author's profile & publications

Annexe I Photodocumentation

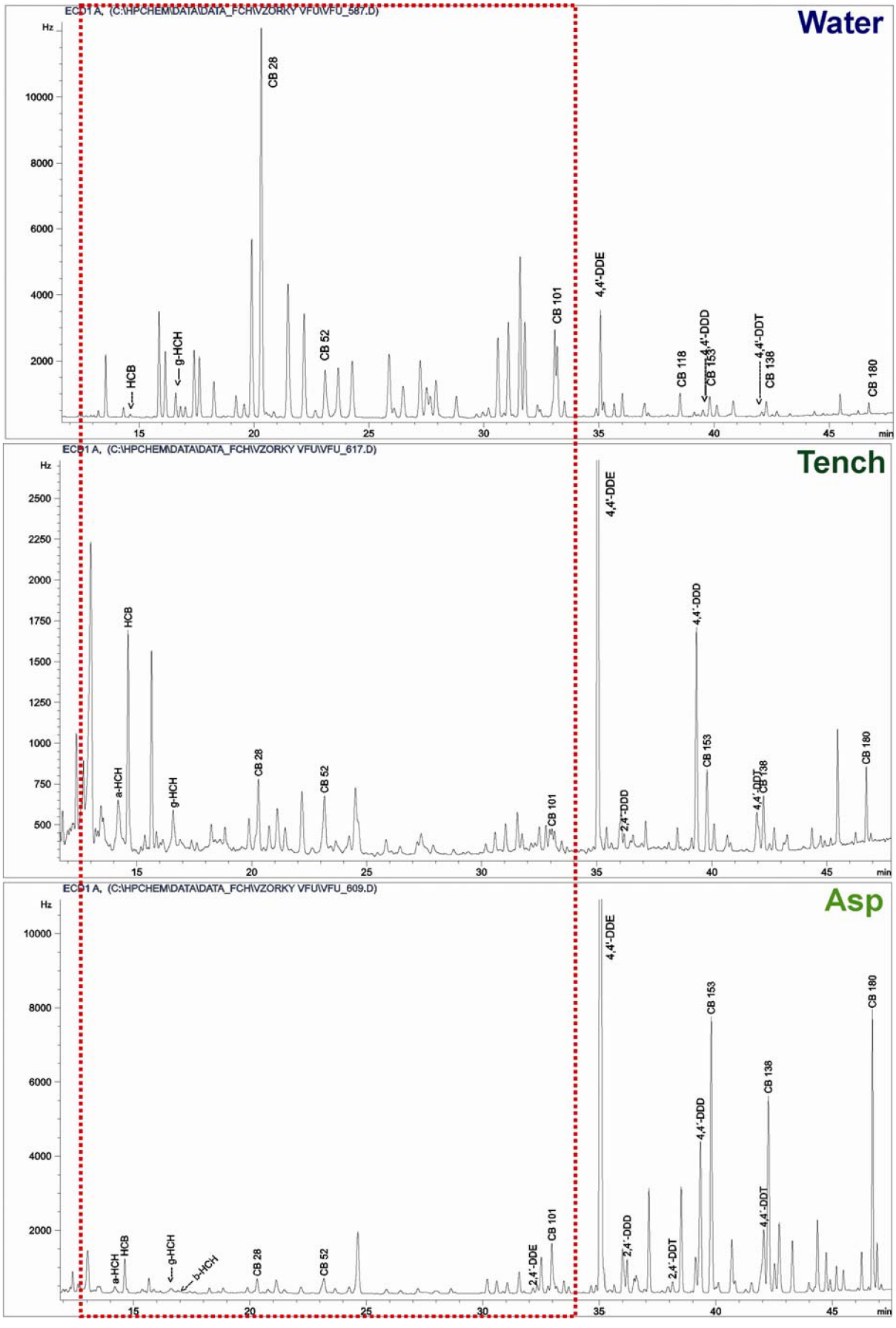


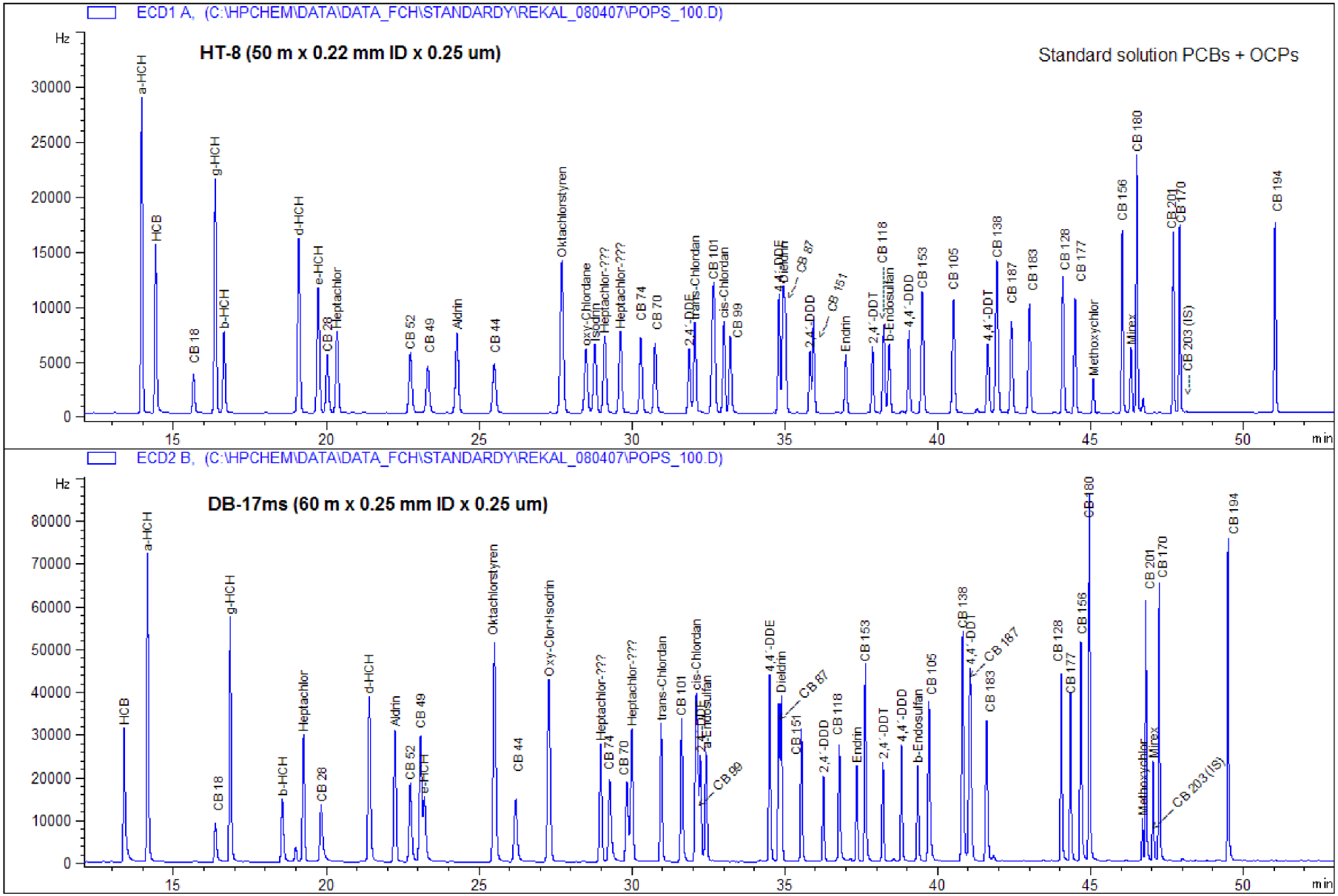



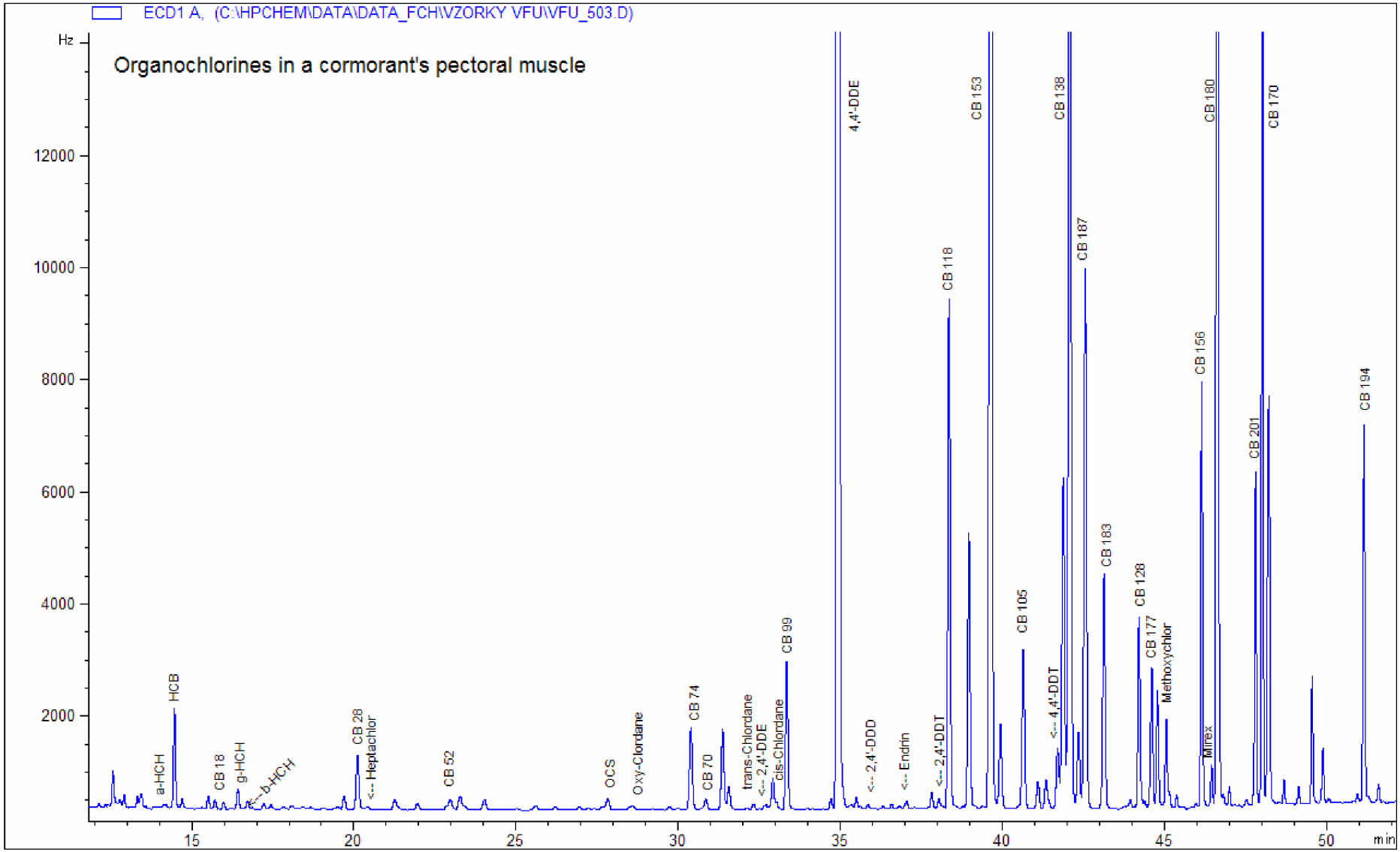
From top left clockwise: (a) fishing by means of electrical device (Svratka River), (b) chub specimen, (c) age determination using scales, (d) age determination

From left: (a) 3-m BP4 sediment core (Brno Lake, 2008), (b) MAE device at FCH BUT (2007)

Annexe II Sample chromatograms (pattern changes in samples from Brno Lake)




ECD1 A, (C:\HPCHEM\DATA\DATA_FCH\STANDARDY\REKAL_080407\POPS_100.D)
HT-8 (50 m x 0.22 mm ID x 0.25 um)
Standard solution PCBs + OCPs
Hz
min
a-HCH
HCB
g-HCH
b-HCH
d-HCH
e-HCH
Heptachlor
CB 18
CB 28
CB 52
CB 49
Aldrin
CB 44
Oktachlorstyren
oxy-Chlordane
Isodrin
Heptachlor-???
CB 74
CB 70
2,4'-DDE
trans-Chlordan
CB 101
cis-Chlordan
CB 99
4,4'-DDE
Dieldrin
CB 87
2,4'-DDD
CB 151
Endrin
2,4'-DDT
CB 118
b-Endosulfan
4,4'-DDD
CB 153
CB 105
4,4'-DDT
CB 138
CB 187
CB 183
CB 128
CB 177
Methoxychlor
CB 156
CB 180
Mirex
CB 201
CB 170
CB 203 (IS)
CB 194
ECD2 B, (C:\HPCHEM\DATA\DATA_FCH\STANDARDY\REKAL_080407\POPS_100.D)
DB-17ms (60 m x 0.25 mm ID x 0.25 um)
Hz
min
HCB
a-HCH
g-HCH
CB 18
b-HCH
Heptachlor
CB 28
d-HCH
Aldrin
CB 52
CB 49
e-HCH
Oktachlorstyren
CB 44
Oxy-Clor+Isodrin
Heptachlor-???
CB 74
CB 70
Heptachlor-???
trans-Chlordan
CB 101
cis-Chlordan
a-Endosulfan
CB 99
4,4'-DDE
Dieldrin
CB 151
CB 87
2,4'-DDD
CB 118
Endrin
CB 153
2,4'-DDT
4,4'-DDD
b-Endosulfan
CB 105
CB 138
4,4'-DDT
CB 187
CB 183
CB 128
CB 177
CB 156
CB 180
CB 201
CB 170
Methoxychlor
Mirex
CB 203 (IS)
CB 194


ECD1 A, (C:\HPCHEM\DATA\DATA_FCH\VZORKY VFU\VFU_503.D)
Hz
Organochlorines in a cormorant's pectoral muscle
12000
10000
8000
6000
4000
2000
15
20
25
30
35
40
45
50
min
a-HCH
HCB
CB 18
g-HCH
b-HCH
CB 28
<-- Heptachlor
CB 52
OCS
Oxy-Clordane
CB 74
CB 70
trans-Chlordane
<-- 2,4'-DDE
cis-Chlordane
CB 99
4,4'-DDE
<-- 2,4'-DDD
<-- Endrin
<-- 2,4'-DDT
CB 118
CB 153
CB 105
<-- 4,4'-DDT
CB 138
CB 187
CB 183
CB 128
CB 177
Methoxychlor
Mirex
CB 156
CB 180
CB 201
CB 170
CB 194

Annexe III Concentrations of PCBs in tissues of selected cormorants – Záhlinice Ponds (ng/g wet weight)
(The values are presented as **median** (min-max) for n = 8 specimens, sums presented as Σ of **means** (Σ of medians)).

	Pectoral muscle	Femoral muscle	Heart	Kidney	Liver	Skin	Brain	Intestinal contents	Feathers
Fat[+] (%, m/m)	2.63 ± 3.06	1.88 ± 1.46	2.38 ± 1.34	3.34 ± 2.26	1.55 ± 0.88	20.29 ± 14.8	6.71 ± 1.46	4.09 ± 2.73	1.94 ± 1.76
CB 18	**1.1** (0.4-3.8)	**1.3** (0.1-2.8)	**1.0** (0.1-2.4)	**1.0** (0.3-3.1)	**0.8** (0.4-1.6)	**11.7** (0.6-21.7)	**1.1** (0.6-5.2)	**4.7** (1.5-17.0)	**4.4** (1.0-40.9)
CB 28	**3.3** (1.4-6.6)	**3.0** (0.3-9.8)	**3.6** (0.5-5.0)	**2.2** (1.4-6.6)	**2.5** (0.7-5.3)	**41.4** (1.7-61.8)	**2.5** (1.8-11.9)	**9.4** (2.9-46.5)	**9.0** (2.2-127.3)
CB 44	**0.4** (0.1-1.0)	**0.4** (0.1-0.9)	**0.4** (0.1-0.6)	**0.3** (0.1-0.8)	**0.3** (0.1-0.4)	**3.8** (0.2-8.6)	**0.4** (0.3-3.5)	**1.6** (0.4-11.3)	**1.6** (0.4-35.8)
CB 49	**0.3** (0.1-1.0)	**0.3** (0.1-0.7)	**0.3** (0.1-0.6)	**0.4** (ND-0.8)	**0.2** (0.1-0.4)	**4.0** (0.1-13.5)	**0.4** (0.2-2.5)	**1.2** (0.4-9.3)	**1.6** (0.3-30.0)
CB 52	**0.7** (0.2-1.9)	**0.6** (0.1-1.1)	**0.4** (0.2-1.0)	**0.8** (0.1-1.7)	**0.4** (0.1-0.8)	**7.0** (0.9-14.5)	**0.5** (0.3-2.6)	**3.7** (0.6-9.5)	**3.7** (0.7-34.7)
CB 70	**0.8** (0.2-1.9)	**0.7** (0.2-1.5)	**0.6** (0.2-1.4)	**0.8** (0.2-2.1)	**0.5** (0.3-0.8)	**6.1** (0.8-17.4)	**1.5** (0.3-5.5)	**2.6** (0.4-22.3)	**5.1** (1.0-47.7)
CB 74	**1.3** (0.6-6.3)	**1.0** (0.2-7.1)	**1.2** (0.3-3.3)	**1.1** (0.3-2.7)	**0.8** (0.1-7.1)	**11.6** (1.0-91.0)	**0.7** (0.3-3.8)	**2.9** (0.7-14.8)	**2.8** (0.4-34.8)
CB 99	**1.3** (0.9-11.5)	**1.5** (0.3-12.7)	**2.9** (0.4-6.8)	**2.2** (0.1-4.2)	**1.6** (0.1-11.9)	**14.4** (2.3-145.4)	**0.9** (0.2-4.6)	**1.8** (0.2-5.5)	**2.4** (0.3-7.9)
CB 101	**0.6** (0.2-2.5)	**0.8** (0.2-3.3)	**0.8** (0.2-1.9)	**0.7** (0.3-1.6)	**0.6** (0.2-1.1)	**6.2** (0.7-32.5)	**0.5** (0.2-1.1)	**2.1** (0.3-13.4)	**2.0** (0.2-45.1)
CB 105	**0.9** (0.5-9.0)	**1.2** (0.2-9.9)	**1.8** (0.3-4.1)	**1.3** (0.1-2.3)	**1.2** (0.1-10.6)	**7.9** (1.4-103.0)	**0.7** (0.1-3.2)	**2.2** (0.2-3.8)	**1.8** (0.2-5.6)
CB 118	**3.1** (1.9-39.0)	**3.8** (0.7-42.6)	**6.0** (0.9-17.4)	**4.8** (0.2-10.0)	**3.6** (0.3-39.7)	**30.6** (5.9-477.6)	**2.0** (0.4-14.1)	**4.8** (0.4-13.8)	**5.2** (0.6-22.8)
CB 128	**0.9** (0.5-10.1)	**1.1** (0.2-10.5)	**1.6** (0.2-4.6)	**1.3** (ND-2.3)	**1.1** (0.1-10.0)	**8.7** (1.5-106.3)	**0.5** (0.1-2.8)	**1.1** (ND-2.8)	**1.5** (0.2-5.0)
CB 138	**5.7** (3.4-82.3)	**5.7** (1.2-89.0)	**9.4** (1.7-34.9)	**7.8** (0.2-19.1)	**5.4** (0.5-80.9)	**57.1** (7.4-979.1)	**2.5** (0.4-25.4)	**4.5** (0.4-21.2)	**7.2** (0.8-37.6)
CB 153	**10.3** (5.5-195.2)	**9.2** (2.4-194.2)	**15.4** (3.9-76.2)	**12.9** (0.5-40.9)	**8.0** (0.8-158.0)	**97.2** (12.9-2462.7)	**5.0** (0.8-57.3)	**8.1** (0.8-44.4)	**12.5** (1.5-79.1)
CB 156	**0.9** (0.4-16.6)	**1.0** (0.3-17.6)	**1.3** (1.3-6.8)	**0.9** (ND-3.6)	**0.8** (0.1-14.3)	**6.9** (1.1-179.2)	**0.3** (0.1-4.9)	**0.6** (0.2-3.8)	**1.1** (0.1-5.8)
CB 170	**1.8** (0.7-34.2)	**1.5** (0.4-36.0)	**2.0** (0.5-13.0)	**1.4** (0.1-6.8)	**1.0** (0.2-28.6)	**10.4** (1.8-374.3)	**0.6** (0.1-9.6)	**1.0** (0.2-6.3)	**1.4** (0.2-11.0)
CB 177	**1.8** (0.3-9.0)	**0.7** (0.7-9.8)	**1.4** (0.2-3.9)	**1.1** (ND-2.2)	**1.2** (0.2-13.0)	**6.8** (0.7-123.0)	**0.3** (0.1-2.9)	**0.7** (0.2-2.2)	**1.0** (0.1-4.8)
CB 180	**5.4** (2.0-119.3)	**3.1** (1.4-119.6)	**5.6** (1.2-43.7)	**4.8** (0.3-21.9)	**2.7** (0.3-90.4)	**35.9** (3.3-1470.4)	**1.6** (0.3-32.0)	**2.8** (0.4-19.0)	**4.1** (0.5-35.2)
CB 183	**1.1** (0.4-14.9)	**0.8** (0.3-15.8)	**1.4** (0.3-6.1)	**1.0** (0.1-3.3)	**0.6** (0.1-12.4)	**7.8** (0.7-173.5)	**0.4** (0.1-4.7)	**0.7** (0.2-3.4)	**0.9** (0.1-5.8)
CB 187	**3.3** (1.1-43.0)	**2.2** (0.6-45.3)	**4.5** (0.8-18.0)	**3.4** (0.2-9.8)	**1.8** (0.3-38.4)	**22.7** (2.3-591.3)	**1.0** (0.2-14.5)	**1.9** (0.2-10.2)	**2.9** (0.4-20.0)
CB 194	**0.7** (0.2-13.8)	**0.6** (0.2-12.5)	**0.7** (0.3-4.8)	**0.5** (ND-2.6)	**0.4** (0.1-9.3)	**4.1** (0.6-184.9)	**0.2** (0.1-3.2)	**0.3** (ND-1.9)	**0.6** (0.1-3.9)
CB 201	**0.9** (0.2-13.5)	**0.6** (0.2-12.9)	**1.0** (0.2-5.1)	**0.7** (0.1-2.7)	**0.4** (0.1-10.3)	**6.3** (0.6-203.9)	**0.2** (0.1-3.8)	**0.4** (0.1-7.3)	**0.7** (0.1-5.3)
Σ22 PCB	**133.0** (45.7)	**146.8** (40.9)	**99.3** (63.5)	**65.4** (51.4)	**98.7** (35.7)	**1373.4** (408.5)	**50.0** (23.9)	**99.4** (58.8)	**140.6** (73.2)

[+] average value + SD; ND – not detected

Annexe IV Concentrations of organochlorine pesticides in tissues of selected cormorants – Záhlinice Ponds (ng/g wet weight)
(The values are presented as **median** (min-max) for n = 8 specimens, sums presented as Σ of **means** (Σ of medians)).

	Pectoral muscle	Femoral muscle	Heart	Kidney	Liver	Skin	Brain	Intestinal contents**	Feathers
Fat[+] (%, m/m)	2.63 ± 3.06	1.88 ± 1.46	2.38 ± 1.34	3.34 ± 2.26	1.55 ± 0.88	20.29 ± 14.8	6.71 ± 1.46	4.09 ± 2.73	1.94 ± 1.76
α-HCH	- (ND-0.1)	- (ND-0.6)	- (ND-0.1)	- (ND-0.1)	- (ND-0.1)	**0.3** (ND-0.6)	**0.1** (ND-0.2)	**0.1** (ND-0.2)	**0.1** (ND-0.4)
β-HCH	**0.4** (0.1-1.0)	**0.5** (ND-0.9)	**0.4** (0.1-1.4)	**0.2** (0.1-0.5)	**0.3** (0.1-0.8)	**1.8** (1.2-13.5)	**0.1** (ND-0.5)	**0.9** (ND-2.7)	**0.9** (0.1-1.4)
γ-HCH	**0.5** (0.1-1.3)	**0.5** (ND-1.2)	**0.4** (ND-1.2)	**0.3** (0.1-1.0)	**0.3** (0.1-0.6)	**3.9** (0.3-10.9)	**0.5** (0.3-1.7)	**1.9** (0.7-13.1)	**2.8** (0.8-7.6)
Σ HCHs	**1.1** (0.9)	**1.1** (1.0)	**1.0** (0.9)	**0.7** (0.5)	**0.7** (0.6)	**10.9** (6.1)	**1.0** (0.6)	**4.8** (2.9)	**4.1** (3.8)
4,4'-DDT	**2.8** (1.2-30.0)	**2.4** (0.5-33.1)	**4.4** (0.9-13.6)	**3.2** (0.1-7.6)	**2.4** (0.6-34.5)	**21.4** (3.7-434.0)	**0.8** (0.1-9.7)	**1.9** (0.2-9.0)	**3.5** (0.8-18.4)
4,4'-DDE	**50.2** (21.1-592.4)	**47.1** (10.8-623.3)	**104.7** (17.7-240.3)	**77.4** (1.6-136.2)	**60.5** (2.4-529.2)	**553.9** (88.6-9507)	**23.6** (2.1-137.6)	**22.4** (2.0-128.1)	**62.7** (25.7-324.2)
4,4'-DDD	**0.3** (ND-1.1)	**0.3** (ND-0.5)	**0.2** (ND-0.9)	**0.2** (ND-0.5)	**0.1** (ND-0.5)	**1.6** (0.3-3.4)	**0.1** (ND-0.3)	**0.9** (0.1-2.9)	**1.0** (0.4-3.0)
2,4'-DDT	**0.2** (0.1-1.0)	**0.1** (ND-1.1)	**0.2** (ND-0.5)	**0.1** (ND-0.3)	**0.1** (ND-0.2)	**1.2** (ND-12.8)	**0.1** (ND-0.6)	**0.3** (ND-1.6)	**0.4** (0.2-0.9)
2,4'-DDE	**0.1** (ND-0.3)	**0.1** (ND-0.6)	- (ND-0.3)	**0.1** (ND-0.3)	- (ND-0.1)	**0.1** (ND-3.3)	- (ND-0.2)	- (ND-1.2)	**0.2** (ND-3.2)
2,4'-DDD	**0.2** (0.1-0.6)	**0.4** (0.1-2.4)	**0.3** (ND-0.4)	**0.1** (0.1-0.3)	**0.2** (ND-0.5)	**2.0** (0.2-3.6)	**0.1** (0.1-0.6)	**0.5** (ND-2.3)	**0.7** (0.3-3.0)
Σ DDTs	**189.8** (53.7)	**195.1** (50.3)	**140.1** (109.7)	**84.4** (81.1)	**114.9** (63.4)	**1819.7** (580.3)	**40.8** (24.8)	**47.2** (26.0)	**105.6** (68.5)
HCB	**4.9** (0.7-19.1)	**3.8** (0.2-18.1)	**4.3** (0.6-20.1)	**3.5** (0.2-10.2)	**5.4** (0.4-10.6)	**10.0** (2.9-127.2)	**2.0** (0.7-4.6)	**2.5** (0.3-8.4)	**1.9** (0.3-4.1)
OCS	**0.2** (ND-0.8)	**0.1** (ND-0.9)	**0.2** (ND-1.7)	**0.1** (0.1-0.8)	**0.3** (ND-0.8)	**1.0** (0.2-10.3)	**0.1** (ND-0.3)	- (ND-0.3)	**0.1** (ND-0.4)
Heptachlor	**0.1** (ND-0.6)	**0.2** (ND-0.5)	**0.1** (ND-0.5)	**0.1** (ND-0.4)	**0.1** (ND-0.2)	**0.6** (ND-3.7)	**0.1** (ND-0.7)	**0.7** (0.2-2.7)	**0.6** (0.2-0.8)
α-Chlordane	**0.1** (ND-0.8)	*	**0.3** (ND-0.5)	**0.1** (ND-0.3)	**0.1** (ND-0.4)	**0.6** (ND-6.2)	**0.2** (ND-0.9)	**0.9** (ND-15.8)	**1.3** (ND-3.3)
γ-Chlordane	**0.1** (ND-0.2)	*	**0.1** (ND-0.3)	**0.1** (ND-0.2)	- (ND-0.2)	**0.7** (ND-3.7)	- (ND-0.2)	**0.6** (ND-1.6)	**0.4** (0.2-2.4)
oxy-Chlordane	**0.3** (0.1-1.1)	*	**0.2** (ND-0.5)	**0.4** (ND-0.9)	**0.2** (0.1-0.6)	**1.7** (0.6-7.5)	**0.2** (0.1-1.3)	**0.8** (0.1-4.3)	**0.7** (0.3-11.3)
Mirex	**0.3** (0.1-4.5)	**0.5** (ND-5.0)	**0.6** (ND-2.0)	**0.5** (ND-1.1)	**0.4** (ND-3.6)	**3.7** (0.7-62.4)	**0.2** (ND-1.4)	**0.2** (ND-6.8)	**0.7** (0.1-2.4)
Methoxychlor	**0.1** (ND-2.2)	**0.2** (ND-2.9)	**0.3** (ND-0.9)	**0.2** (ND-0.5)	**0.1** (ND-1.9)	**0.7** (0.2-35.8)	**0.1** (ND-0.1)	**0.3** (ND-15.1)	**1.1** (0.1-6.3)

[+] average value + SD; * - not evaluated; ** - n = 6; ND – not detected

DISTRIBUTION PATTERNS OF ORGANIC POLLUTANTS IN BRNO RESERVOIR WITH RESPECT TO THE DEPOSITIONAL HISTORY

Eva Franců[1], Jan Schwarzbauer[2], Mathias Ricking[3], Radim Lána[1], Pavel Müller[1], Juraj Franců[1], Daniel Nývlt[1], Slavomír Nehyba[4]

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OBJECTIVES

1. To document the pollution history for the past 70 yrs evidenced in a 3 m sediment profile in the Brno reservoir.
2. To link the pollution events with sedimentary record.
3. To correlate in detail selected compound groups and their immission history.

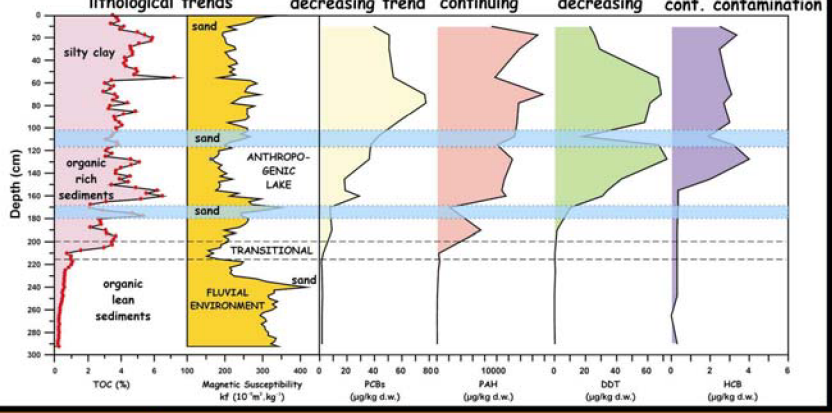
Analytical methods

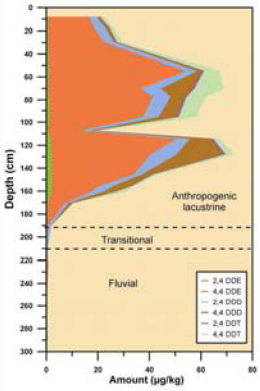
- detail sedimentological description
- magnetic susceptibility (Kf) measured by KLY-2,3 and 3S kappabridges (AGICO a.s.)
- elemental analysis (TOC, TIC)
- GC-MS of PAHs
- GC of PCBs and OCP.

The clayey silts to silty clays in the **upper** 200 cm of the profile were deposited in anthropogenic lake environment. Two sandy interlayers (101-117 and 169-180 cm) are interpreted as flood events.

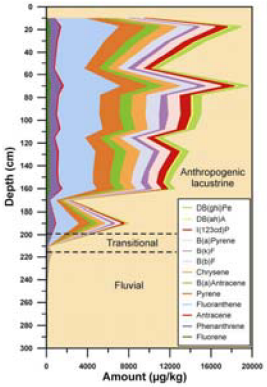
The unusually low values of DDTs and HCB at 107.5 cm and PAH and PCBs at 170 cm are associated with these sandy layers deposited on the site during floods while the clay-contaminant material was transported downstream.

Fluvial sands form **lower** part of the profile (200-296 cm); its uppermost part (200-216 cm) represents the initial filling of the Brno reservoir.

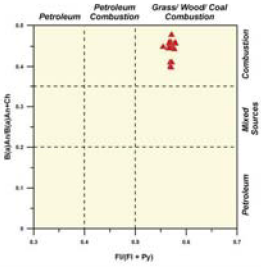


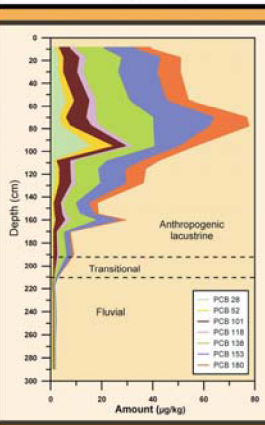


DDT metabolism yields several transformation products. The ratio of DDD and DDE metabolites indicates anaerobic to aerobic environmental conditions and provides information on the kind of microbial activity. Our results suggest more aerobic conditions during DDT transformation, what is an unusual observation in subaquatic sediments.



PAH are useful molecular tool for discrimination of petrogenic and pyrogenic contributions. Brno reservoir PAH occurred at relatively low levels throughout the last 60 years. The correlation (Yunker et al. 2002) of indicative PAH ratios illustrates clearly dominance of pyrogenic matter.





PCBs with the lower or higher degree of chlorination exhibit different fields of application in the technosphere, therefore change in its relative abundance indicates the influence of different emmision sources. In the core samples the Cl6 to Cl7-congeners dominate throughout the profile with exception of the layer around 100 cm, which is enriched in the low chlorinated congeners.

CONCLUSIONS

1. The studied profile archives fluvial, transitional and lake environments as indicated by lithology, TOC and K_f.
2. The geochronological trends of selected priority pollutants show different immission histories partially correlated with periods of their production and application.
3. Short-termed changes in PCB applications are reflected by specific congener ratios.
4. Unusual occurrence of dominant DDE in anaerobic sediments remains contradicts the generally accepted rule.

ACKNOWLEDGEMENTS

Prof. F. Hrouda and Prof. M. Chlupáčová, Agico Ltd. for magnetic susceptibility measurements.

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The Assessment Of The Bioaccumulation Of Selected Halogenated Xenobiotics In The Aquatic Food Chain

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INTRODUCTION

- Organochlorine pollutants (including polychlorinated biphenyls PCBs, organochlorine pesticides OCPs, and dioxines and furanes PCDDs/Fs) are highly persistent, lipophilic and bioaccumulative toxic contaminants formed as a result of either human activities or natural processes. Owing to their specific properties, they persist in the global environment and accumulate in higher trophic levels of the food chains. They are also included among the „dirty dozen" - 12 subgroups of persistent organic pollutants (POPs).
- Some of them have shown to cause cancer and a number of serious non-cancer health effects in animals, including effects on the immune system, reproductive system, nervous and endocrine system and other health effects. E.g. selected PCB congeners have dioxin-related toxic effects.
- The phenomenon of (bio)accumulation and biomagnification of the POPs in the environment has been known for many years and a subject of intensive investigation. Health impacts and toxic effects of POPs are even more serious than the fact of their accumulation itself.
- Owing to the intensive agriculture and industrial production in past few decades, the environment in the Czech Republic is significantly loaded with PCBs and organochlorine pesticides, and their levels are decreasing slowly.

AIMS OF THIS STUDY

- A complex assessment of environmental levels of selected organochlorine POPs in relation to their occurrence in aquatic ecosystems and aquatic food chains by using suitable biomarkers.

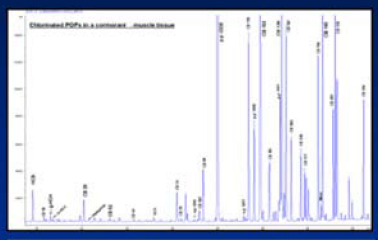
Analytical procedures

- **Sample treatment:** Homogenised fish or cormorant tissues were mixed with anhydrous Na_2SO_4 and extracted for 6 hours with petrolether using a Soxtec extractor. Raw extract were then evaporated to dryness and pure fat was separated. Water samples were extracted with 5 ml of *n*-hexane.
- **Clean-up:** Florisil adsorption columns , elution with hexan-diethylether (94:6), additional treatment with sulphuric acid (if necessary)
- **GC conditions:** HRGC HP 6890N equipped with two μECD detectors, H_2 as carrier gas, N_2 as make-up, **HT-8** (50 m x 0,22 mm i.d. x 0,25 μm film thick. **DB-17MS** (60 m x 0,25 mm i.d. x 0,25 mm film thick). The oven temperature programme: 80 °C – 20 °C/min to 180 °C – 10 °C/min to 250 °C – 2°C/min to 270 °C for 28 min.
- The method detection limits (MDL) for all analytes varied according to the nature of a sample: from 0.03 to 0.3 μg/kg in fish, 0.02 μg/kg in sediments, 0.5 ng/L in water.

RESULTS

- The most abundant compounds determined in muscle tissue of investigated species were 4,4'-DDE, and CB congeners 138, 153, and 180. The greatest mean levels were observed in intestinal contents, then in skin, and in muscle tissue. Naturally, the most 'contaminated' species were predatory fish like asp with 139 μg/kg (Σ7 PCBs) and 349 μg/kg (Σ DDTs), respectively. Other species like bream or chub showed somewhat lower tissue contamination. All levels of POPs found in investigated fish, esp. as far as marketable carps were concerned, were below valid hygienic limits.
- Cormorants (*Phalacrocorax carbo*) lived up to their reputation of being a very greedy and hence the most contaminated member of an aquatic food chain. Since they top this chain, the levels of POPs were higher by almost one order of magn. than those in their fish prey. Moreover, only femoral muscle samples were analysed, thus the levels in other tissues like liver or intestinal content are expected to be by far higher.
- Our results can be well compared with other similar findings not only within the Czech Republic and prove fish to be suitable biomarkers of the contamination of the environment. In relation to other parts of an aquatic food chain (water, sediments, and raptors), the process of accumulation of POPs has been clearly illustrated.

Acknowledgements
Financial support from the Ministry of Education, Youth and Physical Training of the Czech Republic under the research project MSM 6215712402 is greatly appreciated.

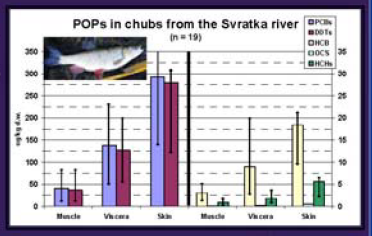


Chromatographic analysis of a cormorant's femoral muscle tissue (GC/ECD)

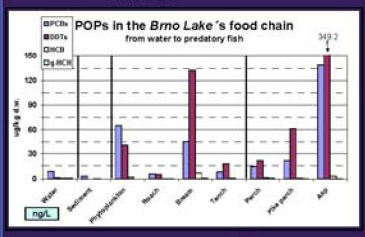
Sampling areas within the Czech Republic

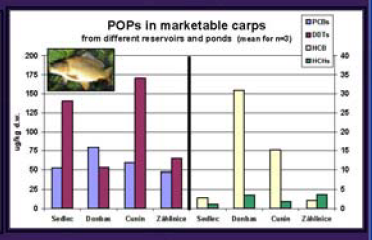
Organochlorine pollutants in aquatic environment

Main Investigated Fish Species



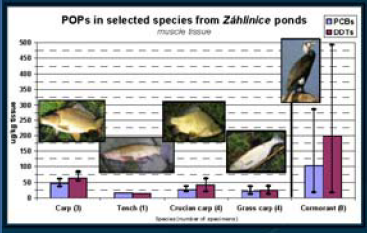
Valid hygienic limits for fish in CR:
PCBs – 2,000 μg/kg, **DDTs** – 500 μg/kg, **HCB** – 50 μg/kg, **γ-HCH** – 50 μg/kg

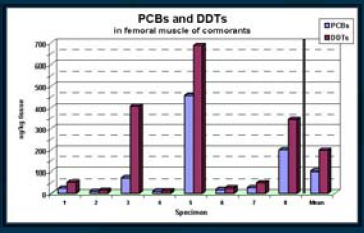


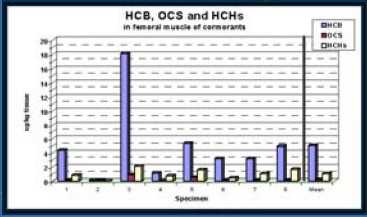


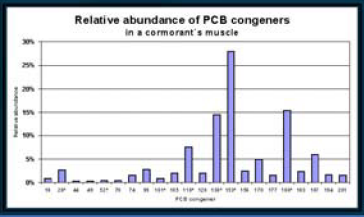
Organochlorine pollutants in CORMORANTS

Besides cormorant specimens, selected fish species caught from the *Záhlinice* ponds were investigated in order to prove the biomagnification of organochlorine pollutants through aquatic food chain – i.e. from fish to a raptor









Annexe VII Author's profile & publications

ABOUT THE AUTHOR

Radim LÁNA (1981) finished his master studies at the Brno University of Technology in 2005 and gained a title of engineer in chemistry and technology of environmental protection. His diploma thesis, dealing with the issue of polychlorinated biphenyls in river sediments, was awarded a Dean's Prize. In the same year he started his doctoral studies of the environmental chemistry and since September 2007 he has been working in the Laboratory of Organic Geochemistry of the Czech Geological Survey as an analytical chemist specialising in the field of GC and MS analyses for the determination of organic pollutants in the environment.

PUBLICATIONS

(September 2005 - October 2008)

Articles

- **Lána, R.,** Šucman, E., Vávrová, M., Mášová, M.: *Comparison of microwave-assisted extraction and pressurised solvent extraction for the determination of persistent organic pollutants in various matrices.* Inter. Journal of Environ. Anal. Chem. (2008), xxxx:xx-xx ***(accepted for peer-review)***

- **Lána, R.**, Vávrová, M., Čáslavský, J., Skoumalová, M., Bílková, A. & Šucman, E.: *PCBs in samples from the environment of the Southern Moravia region, Czech Republic*, Bull. of Environ. Contam. Toxicol. (2008) DOI: 10.1007/s00128-007-9189-4 ***(In press)***

Lectures

- **Lána, R.**, Vávrová, M., Večerek, V., Kráčmar, S.: *Tissue-specific distribution and accumulation of organochlorine pollutants in selected raptor species from the Czech Republic.* The 4th Meeting on Chemistry & Life, Brno, Czech Republic, 9. – 11. 9. 2008

- Vávrová, M., **Lána, R.,** Hroch, M., Hlaváčková, I., Čáslavský, J., Tremlová, B.: *The using of pine-needles, bryophytes and sponges as bioindicators for the evaluation of persistent organic pollutants environmental levels.* The 4th Meeting on Chemistry & Life, Brno, 9.-11. 9. 2008

- **Lána, R.**, Franců, J., Franců, E.: *Organic Geochemistry and Sediment Analysis: Methods and Applications in Mapping and Environmental Quality Monitoring.* Workshop for Geological Survey of Iran, Tehran, Iran, 2. – 3. 9. 2008

- **Lána, R.**: *Plynová chromatografie a její využití při stanovení organochlorovaných polutantů v životním prostředí.* Czech Geological Survey, Brno, 12. 12. 2007

- **Lána, R.**, Vávrová, M., Večerek, V., Palíková, M.: *The bioaccumulation of organochlorine pollutants in various ecosystems*. Conference of PhD students at the Faculty of Chemistry, BUT, Brno, 22. 11. 2007

Posters

- ✓ Franců, E., Schwarzbauer, J., Ricking, M., **Lána, R.**, Müller, P. & Franců, J.: *Distribution patterns of organic pollutants in Brno Lake with respect to its depositional history.* The 4th Meeting on Chemistry & Life, Brno, Czech Republic, 9. – 11. 9. 2008

- ✓ Vávrová, M., **Lána, R.**, Navrátil, S., Večerek, V., Brabencová, E.: *Organochlorine pollutants in chubs from the surface water*. The 32th International Symposium on Capillary Chromatography, Riva del Garda, Italy, 27. – 30. 5. 2008

- ✓ Vávrová, M., Stoupalová, M., **Lána, R.**, Čáslavský, J., Večerek, V.: *Selected congeners of polychlorinated biphenyls in water from several dam lakes in Morava.* The 32th International Symposium on Capillary Chromatography, Riva del Garda, Italy, 27. – 30. 5. 2008

- ✓ **Lána, R.**, Vávrová, M. & Hroch, M.: *The assessment of the bioaccumulation of selected halogenated xenobiotics in the aquatic food chain*. The 8th European Meeting on Environmental Chemistry, Inverness, Scotland, 5. – 8. 12. 2007
  (Best Poster Presentation Award)

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