Stockholm University

Chemical reactivities as a mirror of environmental transformations - method development and assessment of some selected organohalogens

Lisa Granelli

Department of Material and Environmental Chemistry
Stockholm University

Stockholm 2011

Doctoral Thesis 2011
Department of Material and Environmental Chemistry
Stockholm University
SE-106 91 Stockholm
Sweden

Abstract

The assement of chemical persistence is an important part of legislative protection of the environment and human health. Of the vast number of chemicals on the market today few have been properly assessed. The coordination between testing guidelines from different frameworks is limited and especially the methods for determination of biodegradation show poor reproducibility because of their highly complex nature.

In order to circumvent the multifactorial assessment methods that involve the use of e.g. soils and sediments an attempt to create a new approach to chemicals assessment was postulated by Green and Bergman in 2005. This approach puts the focus on testing the chemical reactivity of the compound in environmentally relevant transformation/degradation reactions, i.e. reduction, oxidation, hydrolysis-substitution-elimination (hse), radical reactions, and photolysis. These tests are to be performed in controlled abiotic laboratory experiments ensuring that the results reflect the transformation rate of the intended type of reaction for the investigated substance. To achieve an assessment of the presistence of the compound, the test results are then combined with data on physicochemical properties of the compound and a mathematic matrix describing the reactive power of the different types of reactions in each environmental compartment (air, water, soil, and sediment).

Thus far methods for testing of oxidation, photolysis, and hydrolysis-substitution-elimination reactions have been developed. Within this thesis a method for determining reduction was developed and further utilised to determine transformation products from reductive debromination of the three nonabrominated diphenyl ethers. The previously established method for hse was evaluated and further developed in a study of selected chlorobenzenes. Some novel brominated flame retardants were investigated using the previously developed photolysis method, and transformation products and quantum yields were determined. All of the papers presented within this thesis intend to build on the project of a new persistency assessment model. The results presented also contributes important information on the properties and transformation of some common organohalogen pollutants.

ISBN 978-91-7447-299-8
Universitetsservice US-AB, 2011

Table of contents

Abbreviations

1,2,4,5-TeCB	1,2,4,5-Tetrachloro-benzene
1,2,4-TCB	1,2,4-Trichlorobenzene
BEH-TBP	(2-Ethylhexyl) tetrabromophthalate
BFRs	Brominated flame retardants
BOD	Biochemical oxygen demand
BTBPE	1,2-Bis(2,4,6-tribromophenoxy)ethane
CMR	Carcinogenic-, mutagenic-, and compounds toxic for reproduction
COD	Chemical oxygen demand
CSAs	Chemical safety assessments
DBDPE	Decabromodiphenyl ethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DMF	Dimethylformamide
DOC	Dissolved organic carbon
ECNI	Electron-capture negative ion
EH-TBB	2-Ethylhexyl 2,3,4,5-tetrabromobenzoate
EI	Electron ionisation
HBCDD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HR-MS	High resolution-mass spectrometry
Hse	Hydrolysis, elimination, and substitution
NAS	Nucleophilic aromatic substitution
NDIR	Nondispersive infrared
OECD	Organisation for economic co-operation and development
PBDEs	Polybrominated diphenyl ethers
PBiT	Persistent, bioaccumulating and inherently toxic
PBT	Persistent, bioaccumulative, and toxic
PCBs	Polychlorinated biphenyls
PeCB	Pentachlorobenzene
POPs	Persistent organic pollutants
RBT	Ready biodegradability test
REACH	Registration, evaluation and authorisation of chemical substances
SIM	Selected ion monitoring
STP	Sewage treatment plant
SVHC	Substances of very high concern
TBBPA	Tetrabromobisphenol A
TBBPA-dbpe	Tetrabromobisphenol A bis(2,3-dibromopropyl ether)
$ThCO_2$	Theoretical carbon dioxide
ThOD	Theoretical oxygen demand
TSCA	Toxic substances control act
TSMP	Toxic substances management policy
vPvB	Very persistent and very bioaccumulative

List of papers

This thesis is based on the following papers, which are referred to in the text by their roman numerals, I-IV. Papers II and III are reproduced with the kind permission of the publisher. Paper I is a submitted manuscript and Paper IV is a manuscript.

I Granelli L., Eriksson J., Bergman Å. Sodium borohydride reduction of individual polybrominated diphenyl ethers. *Submitted manuscript.*

II Granelli L., Eriksson J., Athanasiadou, M., Bergman Å. Reductive debromination of nonabrominated diphenyl ethers by sodium borohydride and identification of octabrominated diphenyl ether products. (2011). Chemosphere, 82, 839-846.

III Qiu, Y., Eriksson J., Granelli, L., Bergman Å. An improved method for assessing relative nucleophilic substitution reactivities of polychlorinated benzenes. (2009). Chemosphere, 75, 78-82.

IV Granelli L., Eriksson J., Bergman Å. Photolysis of four novel brominated flame retardants. *Manuscript.*

1 Introduction

1.1 Background

1.1.1 Chemicals industry development

The chemicals industry is essential to our world economy with an overall world sale of €1,871 billion in 2009 [1]. Most of its products are truly important and beneficial innovations for our lifestyle, e.g. new materials, fuels, fertilisers and intermediates for further chemical synthesis. However, some of the building stones for these expressions of human innovation are chemicals that come into use before we are fully aware of their properties, other than their efficiency in achieving the purpose we intend them for.

A classic, well-known example of detrimental side-effects of large scale use of chemicals is dichlorodiphenyltrichloroethane (DDT) [2]. The use of this pesticide, starting on a larger scale during the Second World War, increased and peaked with a production volume of 81,154 tonnes in 1963 [3]. However, in the 1960s DDT was linked to a decline of certain bird populations, notably birds of prey such as the White-tailed Sea Eagle (*Haliaeetus albicilla*) [4,5]. Dichlorodiphenyldichloroethylene (DDE, a transformation product of DDT), accumulated in the bodies of the birds causing reproductive effects, primarily eggshell thinning. Other studies have shown that DDT and its metabolites may also have detrimental effects on the human health [6-8]. In most developed countries the use of DDT was subsequently banished during the 1970s and 1980s and is now subjected to regulations in accordance with the Stockholm Convention on persistent organic pollutants (POPs) [9]. Disease vector control is still permitted as a public health exemption against mosquitoes spreading e.g. malaria. Eleven countries in Africa, seven in Asia, and five in Latin America still use DDT for this purpose and the World Health Organisation (WHO) endorses the use of DDT in indoor residual spraying to combat the disease [2,10]. DDT is hence still manufactured and is used for other pesticidal purposes, besides in malaria prevention, in e.g. China, India, and Bangladesh and possibly other countries as well [11-14].

Polychlorinated biphenyls (PCBs) are a group of industry chemicals that came into production in 1929 [15]. They were used in a variety of applications, e.g. as dielectric fluids in capacitors, coolants and transformers [15]. The toxic properties of PCBs were discovered quite early on as Drinker and co-workers presented evidence of liver damage caused by inhalation of PCB vapour or through skin contact [16]. The study was initiated as a number of workers at the PCB production companies Halowax and General Electric developed what was to be called “chloracne” and two diseased workers were found to have suffered from severe liver damage [16,17]. Production of PCBs continued

none the less. In 1966 Sören Jensen detected PCBs in marine animals, revealing their alarming persistent and bioaccumulative properties [18,19]. PCB toxicity varies between congeners: Baring structural similarities to dioxins, the non-*ortho* and mono-*ortho* substituted PCB congeners, also share their toxicological effects including dermal toxicity, immunotoxicity, carcinogenicity, and reproductive and developmental toxicity [20,21]. Others show neurotoxicological or endocrine disruptive properties [20]. In 1968 the outbreak of a skin disease in the Japanese town of Kyushu, and the subsequent investigation, revealed a PCB contamination in rice oil. PCB containing heat mediator oil leaked into a tank of rice oil and some 2,000 people were affected [20]. As the oil was heated some of the PCB congeners formed the even more toxic polychlorinated dibenzofurans [20]. Some 30 years after the end of PCB production it is still an issue [22,23]. It seeps out of old materials and articles containing PCBs and the compounds themselves are so resistant to degradation that the presence of PCBs in the environment, and in humans, will continue to be a factor far into the future.

Fire prevention using chemicals of some sort has been in practice since the days of ancient Egypt, around 450 BC, where alum was used to protect wood [24]. In later years, synthetic halocarbons, nitrogen containing compounds and phosphate esters have been added to the fire prevention strategies [25]. The mechanism of halogenated flame retardants is that of gas phase radical quenching and the compound itself is either incorporated into the material it is to protect or introduced as an additive [26]. Organobromine flame retardants have a higher radical trapping efficiency and lower decomposing temperature than their organochlorine counterparts and in 2003 about 25% of the flame retarding products contained bromine [27]. Brominated flame retardants (BFRs), e.g. the most common compounds polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA), have been shown to have undesirable effects in wildlife and humans [28-30]. The acute toxicity of the most common BFRs is low and the products exhibit e.g. developmental neurotoxicity, morphological effects in the liver, and effects on the thyroid hormones [29]. A detailed account of BFR toxicity can be found in the San Antonio Statement on Brominated and Chlorinated Flame Retardants, 2010 [31]. An important point about the BFRs is that their structural properties and chemical composition often make them persistent and bioaccumulative, threatening both environment and human health. The case of the BFRs emphasises an important question: Which effects are acceptable as long as the main purpose of the chemical or product is reached?

1.1.2 Stockholm Convention

The Stockholm Convention on Persistent Organic Pollutants is an international treaty that aims to eliminate or restrict the production and use of

POPs [9]. It has to date been ratified by 172 parties (151 signatories), Russia and the USA notable exceptions [32]. It is the first global, legally binding agreement of its kind with scientifically based criteria for potential POPs. In Annex D of the Convention four evaluation criteria of POPs are defined as being: "Persistence, bio-accumulation, potential for long-range environmental transport and adverse effects" [9]. To this is also added the evaluation criteria: "very persistent and very bio-accumulative" (vPvB) [9], acknowledging the fact that the evaluation of the compounds has more than once been one step behind the evidence of adverse effects in the environment and that this needs to be addressed in legislature. The compounds and products under the Stockholm Convention are listed in Table 1.1.

Table 1.1. Compounds listed as POPs under the Stockholm Convention [9].

Compound(s)	**Use**	**Action**
Aldrin	Pesticide	Elimination
Chlordane	Pesticide	Elimination
Chlordecone	Pesticide	Elimination
Dieldrin	Pesticide	Elimination
Endrin	Pesticide	Elimination
Heptachlor	Pesticide	Elimination
Hexabromobiphenyl	Industrial chemical	Elimination
Hexabromo- & heptabromo-diphenyl ether	Industrial chemicals	Elimination
Hexachlorobenzene	Industrial chemical & pesticide	Elimination
Alpha hexachlorocyclohexane	Pesticide	Elimination
Beta hexachlorocyclohexane	Pesticide & by-product	Elimination
Lindane	Pesticide	Elimination
Mirex	Pesticide	Elimination
Pentachlorobenzene	Industrial chemical & pesticide	Elimination
Polychlorinated biphenyls	Industrial chemicals	Elimination
Tetrabromo- & pentabromo-diphenyl ether	Industrial chemicals	Elimination
Toxaphene	Pesticide	Elimination
DDT	Pesticide	Restriction
Perfluorooctane sulfonic acid, its salts & perfluorooctane sulfonyl fluoride	Industrial chemicals	Restriction
Polychlorinated dibenzo-*p*-dioxins (PCDD)	By-products	Unintentional production
Hexachlorobenzene (HCB)	By-product	Unintentional production
Polychlorinated biphenyls (PCB)	By-products	Unintentional production

Precaution is becoming an elementary basis of environmental legislature today, for instance within the European Union [33,34]. This precautionary principle implies a shift in the burden of proof from government to industry; those promoting a product or activity have to show that it is *not* harmful. Within the European Union this is intended as an incitement to link science, policymakers, and industry tighter together, encouraging feedback and cooperation [35]. There are strong objections to this approach, in particular from the USA [36,37]. In what other context would decision making without substantial evidence be approved? At what level of concern should this principle apply? What could the effects be if the product or activity is not realised? However, the chemicals we use will always to some extent, unless they are highly reactive, end up in our environment and we need to make sure that we limit the damages they can cause. Up until today the burden has lain on our governments to demonstrate harmful effects before taking action. If the responsibility for the testing of chemicals is instead placed on the industry, this can be integrated into their product development and give products of verified safety.

As import and export of goods, migrating fauna and long-range transport via air, particles, and water cancels any notion of regional control, a more restrictive view from as many countries as possible makes restrictions and precautions necessary. In 2003, the European Commission adopted a legislative proposal for a new chemicals policy which was implemented in 2006; the mandatory Registration, Evaluation and Authorisation of CHemical substances (REACH) [38]. The system involves four main sections:

- The registration of chemicals, documentation that risks have been adequately assessed.
- Evaluation of the registration dossiers, mainly considering testing proposals.
- Authorisation of substances of very high concern (SVHC).
- Restriction of substances at the level of the European Community when industry measures are not sufficient.

To aid the chemicals industry in fulfilling their obligations, a REACH Implementation Project has produced technical guidance documents on how to produce chemical safety assessments (CSAs) for all substances manufactured within or imported into the European Union in quantities exceeding 10 tonnes per year. The CSA includes 1) human health hazard assessment, 2) assessment of physico-chemical properties, 3) environmental hazard assessment, and 4) PBT assessment, including identification of vPvB compounds.

The REACH regulation presents criteria for what is termed Substances of Very High Concern (SVHC). Criteria in article 57, a-c, concerns

Carcinogenic, Mutagenic, and compounds toxic for Reproduction (CMR), criteria d-f; persistent, bioaccumulative, and toxic (PBT) and vPvB compounds, plus compounds of so-called equivalent level of concern (e.g. endocrine disruptors).

As the vast majority of chemicals available on the market today have not been thoroughly characterised, this is a monumental task. In order to find the new DDT, PCBs or BFRs before they are released into our environment, we need efficient systems to screen for them.

1.2 Testing

There are a number of regional, national and global conventions aiming to identify and manage POPs and compounds that are PBT to enable protection of human health and the environment [38-41]. At present, there is no official coordination of these approaches and criteria but they show some consistency [42], as presented in Table 1.2.

Table 1.2. Compartmental half-lives ($t_{1/2}$) as POP criteria as postulated by some different regulatory frameworks.

Framework	Criteria
Canada (Toxic Substance Management Programme)	$t_{1/2}$ in air > 2 days, water > 2 months, sediment > 6 months, soil > 1 year
Canada (Canadian Domestic Substances List)	$t_{1/2}$ in air > 2 days, water > 6 months, sediment > 1 months, soil > 6 months
EU (REACH) PBT	$t_{1/2}$ in water > 60 days in marine water, > 40 days in fresh or estuarine water, > 180 days in marine sediment, > 120 days in fresh or estuarine water sediment, > 120 days in soil
EU (REACH) vPvB	$t_{1/2}$ in water > 60 days in marine, fresh or estuarine water, > 180 days in marine, fresh or estuarine water sediment, > 180 days in soil
USA (EPA control action)	$t_{1/2}$ > 2 months
USA (EPA ban pending)	$t_{1/2}$ > 6 months
OSPAR	Non-readily biodegradable or $t_{1/2}$ in water > 50 days

The US Environmental Protection Agency proposes two sets of criteria for PBTs under the Toxic Substances Control Act (TSCA). The criteria of a half-life under two months entails that compounds exceeding this limit has to be subjected to testing and release control. Compounds with half-lives over six months will be banned except if testing can prove that the chemical is not to be considered of "high risk concern" [43]. In Canada, the assessment of persistence under the Toxic Substances Management Policy (TSMP) is based

on criteria for identifying Persistent, Bioaccumulating and Inherently Toxic (PBiT) substances [44].

Japan, with a chemicals sale of €120 billion in 2009 [1], amended their Chemical substances control law in 2009, sharpening the obligations of the manufacturers and importers of existing chemical substances. The amendment came fully into effect April 1, 2011 and one of the purposes with the amendment was to eliminate international inconsistencies and construct rational evaluation and regulation systems in Japan [45].

A common denominator for these approaches is the search for cost efficient test methods that are scientifically based giving as few false negative and false positive results as possible. To tackle the immense task of screening the ever increasing number of chemicals used in society today, a system is needed that allows preliminary screening of chemicals, using robust, standardised tests identifying those chemicals for which more detailed studies are needed.

The guidelines from The Organisation for Economic Co-operation and Development (OECD) for the testing of chemicals provide tools for the assessment of potentially harmful properties and are applied within the European Union. The guidelines include for instance methods for testing the abiotic transformations hydrolysis and photolysis. Further methods include biodegradation in water, soils and sediments effectuated in ready biodegradability tests, simulation tests, inherent biodegradability tests, and anaerobic biodegradability screening tests [46]. In sections 1.2.1-1.2.3, accounts of a selected number of OECD guidelines are given.

1.2.1 Biodegradation

The principal transformation pathway of contaminants in soils and sediments is biodegradation. Biodegradation is the transformation of compounds by microorganisms through reactions such as reduction, oxidation and hydrolysis. The degradative capacity of a soil or sediment is highly dependent on the ambient conditions; modes and rates of biodegradation may vary greatly between different locations [42].

Many of the OECD guidelines for determination of biodegradability involve following the degradation by measurement of gas formation or reduction, or determination of organic carbon content in the reaction vessel.

Dissolved Organic Carbon (DOC) can be used to monitor microbial activity. It can be determined by e.g. high temperature combustion where the inorganic carbon in a sample is converted to dissolved carbon dioxide, and purged from the extracted sample. The remaining (organic) carbon is then oxidised at a high temperature to carbon dioxide which can be detected by e.g. a nondispersive infrared (NDIR) sensor allowing quantification of the carbon content in the sample.

Microorganism activity consumes organic carbon, water and/or oxygen, and produces carbon dioxide, or methane under anaerobic conditions. Oxygen can be determined by different sensors or by registering the partial pressure by the use of an appropriate membrane, carbon dioxide can, as stated above, be monitored by IR-techniques. The amount of oxygen that theoretically can be consumed if a test compound is completely oxidised by microorganisms is defined as the Theoretical Oxygen Demand (ThOD), and by monitoring the actual oxygen level in the test the level of degradation can be determined. Theoretical carbon dioxide ($ThCO_2$) is then the theoretically produced carbon dioxide if the test compound would be completely oxidised.

The microbial activity on an inoculum can be characterised as the Biochemical Oxygen Demand (BOD), which determines the levels of biologically active organic matter, typically by the aid of a pressure sensor. Chemical Oxygen Demand (COD) is the oxygen demand of organic compounds in a water sample and is determined by adding an oxidant to the sample. By titrimetric or spectrophotometric determination of oxidant still remaining in the sample at the end of the test the original carbon content in the sample can be calculated and hence the degradative activity of the microorganisms.

Ready biodegradability tests

The tests proposed by OECD are put forward in a priority order, a strategy designed to be cost efficient, thus avoiding unnecessary laboratory experiments. The first step in the process is screening for so-called ready biodegradability [46]. There are six ready biodegradability tests (RBTs) in the OECD guidelines for testing of chemicals and the choice of method is based on the solubility, volatility and adsorption properties of the test compound.

The RBTs do not provide degradation rates but are constructed to give a pass or a fail result. Passing an RBT indicates that the compound will be degraded or mineralised in most environments. The degradation is followed by measuring either DOC, ThOD or $ThCO_2$.

The tests last 28 days and to pass a 70% removal of DOC and a 60% loss of ThOD or $ThCO_2$ production inside a 10 day window within the 28 day test period is required. The 10 day window starts when the degree of biodegradation has reached 10% DOC, ThOD or $ThCO_2$ and must end before day 28 of the test. An exception to this rule is the MITI test, named after the Japanese Ministry of Trade and Industry from where the test originates, briefly described as follows: A relatively high concentration of the test substance (2-100 mg/L) is used. The source of microorganisms is usually sludge from e.g. activated sludge; sewage effluents (unchlorinated); surface waters and soils; or from a mixture of these. The inoculum should not have

been previously adapted to the test substance or structurally related compounds [47].

In the MITI-I RBT test mentioned above, the diversity of the microorganisms in the sludge is reduced, as well as their ability to acclimate to and degrade various substrates [48]. The substrate is introduced in a concentration of 100 mg/L and the inoculum is 30 mg sludge solids/L. The test lasts for 28 days and if oxygen demand due to degradation of test substance reaches or exceeds 60% of theoretical, the test substance is considered readily biodegradable. The MITI inoculum is prepared by feeding a mixture of sludges from various sources for 30 days with peptone, a mixture of polypeptides and amino acids formed by the partial hydrolysis of proteins, only. These test conditions seem to give less variability in the results than the other RBTs [42].

Inherent biodegradability tests

The inherent biodegradability tests allows extended exposure of the test substance to the microorganisms and a lower ratio of test substance to biomass. This gives a better chance to obtain a positive result, i.e. sufficient biodegradation, compared to tests for ready biodegradability. The tests also include using microorganisms that have previously been exposed to the test substance, something which frequently results in adaptation and a significant increase of the degradation rate. Because of the favourable conditions applied in the inherent tests, rapid biodegradation in the environment of inherently biodegradable chemicals cannot be expected.

Inherent biodegradability is to be considered a specific property of a compound and no limits on test duration or biodegradation rates are defined. Biodegradation above 20% of theoretical (measured as BOD, COD or DOC removal) may be regarded as evidence of inherent, primary biodegradability, whereas biodegradation above 70% of theoretical (measured as BOD, COD or DOC removal) may be regarded as evidence of inherent, ultimate biodegradability.

If a compound fails a test of inherent biodegradability this can lead to a preliminary assumption of potential environmental persistence and testing of potential adverse effects of transformation products that might subsequently be formed in the environment. An alternative interpretation of a negative result in an inherent biodegradability test is that the microorganisms can be inhibited by the test compound. If this can be suspected, the compound can instead be subjected to a simulation test as described in the following section [48-51].

Simulation tests

A chemical that is not readily biodegradable nor shows inherent biodegradability may be rapidly degradable when present at low

concentrations in the environment. The simulation tests are constructed to simulate the specific conditions in environmentally relevant conditions, using e.g. indigenous biomass, surfaces that allow sorption of the chemical, and temperatures representative for a specific environment [51].

There are simulation test guidelines for aerobic biodegradation under environmentally realistic conditions in e.g. surface water, wastewater, and in the aerobic treatment stage of sewage treatment plants (STPs) [52-54].

The test compound is applied in concentrations from less than 1 μg/L to 100 μg/L, the low concentration is intended to provide results that reflect realistic environmental conditions. The biodegradation kinetics are then determined either by monitoring the DOC and/or the COD, using radiolabelled test compounds or by specific chemical analysis.

That the test substance is applied in environmentally realistic concentrations implies that the anticipated biodegradation kinetics are first order ("non-growth" kinetics). Simulation tests can provide e.g. first order or pseudo-first order rate constants, half-lives, and identification of transformation products and their concentrations. In order to enable identification and quantification of degradation products, higher concentrations must be applied. According to the introduction to OECD guidelines, results of simulation tests should only be extrapolated to environmental transformation if the concentrations used in the test are of the same order of magnitude as can be expected in the environment.

In the guidelines for simulation of biodegradation in aerobic sewage treatment plants, no specific pass levels have been defined as the results are assumed to be plant specific, seeing that there are many different plant designs and operating conditions. The test results may be used to estimate the removal in STPs and the resulting effluent concentrations for prediction of the concentration in the treatment plant and the receiving aquatic environment [51].

Aerobic and anaerobic transformation in soil

The OECD guideline aerobic and anaerobic transformation in soil allows for determination of the transformation rate of the test substance and identification of formed products and their transformation rates. Mineralisation rates can be determined by using ^{14}C-labelled start material and trapping the formed $^{14}CO_2$ [55].

The test compound should preferably be added to the soil as a water solution but if necessary a minimum amount of an organic solvent can be added. It is also possible to add the test compound as a solid. The soil samples treated with the test substance are then incubated in the dark in biometer-type flasks or in flow through systems under controlled conditions. For anaerobic test conditions the soil is water-logged and the test system is kept under inert

atmosphere. At appropriate time intervals, soil samples are extracted and analysed for the parent compound and for transformation products. The method is applicable to slightly volatile, non-volatile, water-soluble or water-insoluble compounds. The studies should not exceed 120 days [55].

For studies of transformation rates, the OECD guideline states that at least four types of soils must be used. These should cover a wide range of different organic carbon content, pHs, clay content, and microbial biomass. The soils should further be characterised for other relevant properties such as bulk density, water retention characteristics and cation exchange capacity. The soils should be collected from well-documented locations, including information on e.g. any prior treatment with pesticides or fertilisers. The site should not be used if it has been exposed to the test compound or structurally similar compounds during the last four years [55].

Aerobic and anaerobic transformation in aquatic sediment systems

The upper water phase in an aquatic sediment system is generally aerobic whilst the surface layer of the sediment can be either aerobic or anaerobic and the deepest sediment is usually anaerobic. OECD guidelines provide a test of aerobic transformation in aquatic sediment systems simulating an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient. The anaerobic OECD test simulates a completely anaerobic water-sediment system.

The methods allow measurement of transformation rates in the water-sediment column as well as in the sediment. If a ^{14}C-labelled test compound is used, the rate of mineralisation of the test substance and/or its transformation products can be determined.

Sampling sites for aquatic sediments and sediments should be throughly documented and any history of agricultural, industrial or domestic inputs to the area and the waters upstream must be considered. Sediments should not be used if they have been exposed to the test substance or structurally similar compounds within the previous years. Two different sediments are to be used for the aerobic studies. These should be of fine texture and high carbon content, and coarse texture and low carbon content, respectively. For anaerobic studies, two sediments should be sampled from anaerobic zones of surface water bodies. They should be transported carefully and under inert atmosphere. The sediments should be thoroughly characterised before use. The experiment should continue until the degradation pathway and water/sediment distribution patterns are established or when 90% of the test substance has been removed by transformation and/or volatilisation. The duration of the test should not exceed 100 days. At appropriate sampling times, whole incubation vessels (in replicate) are removed for analysis. Sediment and overlying water are analysed separately [56].

Digestion of sludge refers to the degradation of biomaterials in the sludge by addition of microorganisms. It is amongst other purposes used to process wastewater and to produce biogas [57].

In the OECD test of anaerobic biodegradability in digested sludge, the rate of biodegradation is followed by intermediate measurements of gas production in the reaction vessel. Methane and carbon dioxide production gives an increase in headspace pressure in the reaction vessel. Much of the carbon dioxide will dissolve into the liquid phase and form carbonate or hydrogen carbonate and therefore the inorganic carbon in the primary biodegradation can also be determined by specific analyses at the beginning and end of the test.

The digested sludge should be collected from a waste treatment plant which treats predominantly domestic sewage. An industrial sewage treatment plant can be used as a sludge source if the intention is to use an adapted inoculum. Just prior to use, in order to reduce the inorganic carbon content even further, inoculum should be washed to a concentration of inorganic carbon of less than 10 mg/L in the final test suspension. This can be done in steps of centrifugation, discharge of supernatant and resuspension in de-oxygenated medium until the goal concentration is met, with a maximum of three times. Then the pellet is suspended in the requisite volume of test medium and the concentration of total solids can be determined.

The test compound is applied to the sludge at 20–100 mg C/L in a sealed vessel and is then incubated at 35 ± 2°C for up to 60 days. At least triplicate test vessels for the test substance, blank controls, reference substance inhibition controls and, if desired, pressure control chambers. Additional vessels for the purpose of evaluating primary biodegradation using test substance specific analyses may also be prepared [58].

1.2.2 Hydrolysis

Most hydrolysis reactions follow apparent first order reaction rates and, therefore, half-lives are independent of the concentration of the test compound. The first order kinetics generally allow extrapolation to environmental conditions, of micromolar concentrations, of reaction rates determined in laboratory experiments using much higher concentrations of the test compound [59].

The OECD guidelines for determination of hydrolytic transformations states testing at three different pHs; 4, 7 and 9 [59]. In the environment, sea water has on average a pH of 8 and the span of fresh waters lies between 5 and 8.5. The experiments in the guideline Hydrolysis as a Function of pH are conducted at 50°C. The test compound is introduced as a water solution into sterile buffer solutions of pH 4, 7 and 9. A small amount of water miscible

solvent is allowed but no more than 1% by volume. The degradation experiments are conducted in the dark at constant temperature, under inert atmosphere, and the progress is followed by withdrawing aliquots at appropriate intervals. The test compounds must be sufficiently soluble in water, more than 20 mM, and no more than slightly volatile (undefined). A compound that has undergone less than 10% hydrolytical transformation after five days is considered hydrolytically stable [59].

1.2.3 Photolysis

Photolytical transformation of chemicals in surface water and soil respectively may be examined by use of the guidelines Phototransformation of Chemicals in Water – Direct Photolysis and Phototransformation of Chemicals on Soil Surfaces (draft January 2002) [60,61].

Photolysis will probably be an important transformation pathway for a compound if it exhibits significant light absorption above the 290 nm cut-off of solar irradiation at the earth's surface, the absorption properties of a compound is a result of its chemical structure. The quantum yield for the chemical reaction and of course the solar photon irradiance to which the chemical is exposed also influences phototransformation reactions [61].

Factors such as the physical and chemical properties of the water, latitude and season influences the solar photon irradiance to which a chemical in a water body is exposed. The OECD guideline for Phototransformation of Chemicals in Water – Direct Photolysis enables determination of transformation pathways and identities, concentrations, and rates of formation and decline of phototransformation products. As a light source a filtered xenon arc lamp (to remove irradiation below 290 nm) or sunlight irradiation extrapolated to natural water can be used. The test compound should preferably be dissolved in the test media, buffered water at an appropriate pH, but if necessary, a water miscible solvent can be added, not exceeding 1% by volume. The test should be conducted for a sufficient period of time to determine two half-lives, a maximum of 30 days. A day is defined as 12 hours of light and 12 hours of darkness. If the estimated half-life of the reaction is greater than 30 days, direct phototransformation is considered an insignificant process. Quantum yields and resulting estimated direct photolysis rate constants for the test chemicals for various types of water bodies, seasons, and latitudes can be determined [61].

In the OECD guideline for phototransformation of chemicals on soil surfaces, two thin layers of soil are treated with the test compound and incubated and subjected to simulated sunlight at a controlled temperature. The tests are conducted in flow-through systems in order to trap any volatile products formed during the experiments. A control is kept in the dark to enable distinguishing between photolysis and other types of degradation. Samples from the test soil and the non-irradiated control are extracted and analysed for

the test substance and/or for major phototransformation products. Trapped volatile products are also collected and analysed for at appropriate intervals. Using carbon radiolabelled test compounds, phototransformation products can be identified and a mass balance can be established. The method is applicable to all test substances that are not volatilised from soil [60].

As for all OECD guidelines addressed in this thesis, before performing the tests relevant physico-chemical properties of the compound should be determined, e.g. water solubility and vapour pressure. For the testing of phototransformation on soil and in water, aerobic and anaerobic transformation in soil and phototransformation in water should also be determined beforehand.

Similar to the testing of phototransformation in water, a filtered xenon arc lamp is recommended as light source. The incident light available at the soil surface level, is recorded using a portable spectroradiometer, a device which measures the intensity of radiant energy. These numbers are compared to energy values of natural summer sunlight at midday at latitude 30 to 50° N, obtained from reference or measured with the spectroradiometer. These data are then used to convert the laboratory results into days of natural summer sunlight [60].

One type of soil is sufficient for this test. The selected soil should be characterised regarding texture, pH, organic carbon content and water holding capacity. The soil should be taken from the top layer to a maximum depth of 20cm. Vegetation, macrofauna and stones should be removed. The soil is then dried, desaggregated gently and sieved. Availability of detailed information on the history of the sites from where the test soil is collected is essential. This documentation should include e.g. location, vegetation cover, any previous treatment with crop protection chemicals and/or organic and inorganic fertilisers, as well as details on any accidental contaminations [60].

The test substance should be dissolved in water and then added to the soil thin layer. If necessary an organic solvent can be used but it should be evaporated prior to the experiment. The amount of test substance applied should correspond to a concentration expected of an environmental contaminant, or for crop protection chemicals, to the maximum recommended use rate [60].

1.3 Future of chemical assessment

One conclusion that can be drawn from the tests described above, is that the attempts to create a chemical assessment scheme that imitates environmental conditions has created a quite unwieldy set of time consuming and laboriously intertwined methods.

The use of microorganisms can be viewed as the weakest point in the OECD guidelines. The activity of degrading microbial population and the ability of

the microbes to grow once exposed to a test chemical for instance, as well as a number of other factors, may have great impact on the test results [62]. Furthermore, the reproducibility of the standardised test methods for determination of biodegradability, especially for less reactive species and in interlaboratory comparisons, is often poor [63].

In 2005, Green and Bergman presented a vision of a controlled and robust test system, where the reactivity of the test chemicals can be tested in a non-biotic laboratory environment [64]. If focus is laid on the basic transformation mechanisms, instead of trying to mimic the ways and means of nature to perform these transformations, a more concise chemical assessment can be developed. The results from these laboratory experiments, combined with a matrix describing the reactive power of the environmental compartments the chemicals might end up in, would then give compartmental transformation rates, i.e. predictions of the persistency of the test compound in the environment.

1.4 Aim

The main goal for this thesis was to further develop experimental methodologies to study chemical reactivity of potential environmental contaminants as a new basis for assessment of chemical persistence. A priority was then to identify and describe a method to assess compound susceptibility to reductive transformation under purely abiotic conditions. OctaBDE transformation products from reductive debromination of the three individual nonaBDEs were to be identified to determine any preferential major products from this group of PBDE isomers. Photolysis of some novel brominated flame retardants was performed to point out their chemical reactivity and potential major degradation products. Further, a previously developed method for hydrolysis-substitution-elimination reactions was to be re-evaluated with the goal to improve its correctness and robustness.

2 Transformation chemistry

The methods used in the experiments of this thesis are fundamental chemical transformation processes. The environment contains many reagents and catalysts with action mechanisms ranging from crudeness, as the hydrolysis of esters to carboxylic acids by hydroxyl ions, to the sophistication of induced-fit of substrate binding pockets on proteins. However, to construct methods for testing chemicals for potentially harmful properties, encompassing the entire plethora of naturally occurring degradation phenomena could not be feasible nor would it be practical from a legislature perspective as the testing and evaluation process would reach enormous proportions. The gap that needs

filling is that of practical, robust, relatively inexpensive, and rapid testing. If the general susceptibility of a compound to undergo a certain class of transformation in the environment can be assumed to be reflected in a test of the chemical's tendency to undergo the same transformation type, but by a different agent in a laboratory circumstance, a less complex chemicals assessment can be constructed.

In this section the development of methods to study certain classes of transformation of chemicals within the scope of this thesis are presented, as well as natural occurrences of these degradation pathways and the state of this research field today.

2.1 Reaction rates

The rate of a reaction depends on a number of factors, i.e. temperature, catalysts, and the concentration of reactants. The rate of a one-phase reaction is defined as the disappearance of a reactant or the appearance of a product over a unit of time. As the rate is usually proportional to the reactant concentration, the rate nearly always changes with time as the concentration of reactants decreases.

If the same number of molecules decomposes per unit of time until there are no more molecules the reaction is a zero order rate reaction and can be written as follows:

$$rate = k = -\frac{d[A]}{dt}$$

where *k* is the rate, t is time, and A is the reactant. An example of this type of reaction is the elimination of ingested alcohol.

In a first order rate reaction, the rate is proportional to the change in concentration of only one reactant, an example of this type of reaction is radioactive decay. The half-life of the reaction is constant and the rate can be described as follows:

$$rate = -\frac{d[A]}{dt} = k[A]$$

The rate of a second order reaction is proportional to the concentration of two reactants, or to the square concentration of one:

$$rate = \frac{-d[A]}{dt} = k[A][B] \quad or \quad rate = \frac{-d[A]}{dt} = k[A]^2$$

If e.g. $[A] << [B]$, the concentration of B can be considered to remain constant and the second order rate can be approximated as a pseudo first order reaction. This type of approximation can also be made for example when one

reactant is continuously replenished and when a reaction is conducted in a buffer of some sort.

In assessment of chemical persistence, a pseudo first order approximation of the reaction rate is normally assumed. The reactants are assumed to be in constant excess to the pollutant.

2.2 Reduction

The partition properties of highly halogenated aromatic environmental pollutants make them adsorb to particles and subsequently end up in soils and sediments. As stated in the OECD guideline in Chapter 1.2.1.5, surface layers of sediments can be either aerobic or anaerobic and the deeper sediments are normally anaerobic. Under anaerobic conditions, reductive reactions are common transformation pathways for environmental pollutants and are therefore important to study.

2.2.1 Redox reactions

The chemical term *reduction* cannot be explained without its counterpart *oxidation*. In simple terms, *oxidation* is an increase in the oxidation state/number of a molecule, atom, or ion, often as a result of a loss of one or more electrons. Oxidants (electron acceptors) are usually compounds with elements in high oxidation numbers (e.g., hydrogen peroxide, chromium trioxide and osmium tetroxide) or highly electronegative substances that can gain one or two extra electrons by oxidising compounds containing e.g. oxygen and halogen substituents.

Reduction is then an decrease in oxidation state/number, often the gain of one or more electrons. Reducing agents (electron donors) transfers electrons to another substance, and is thus itself oxidised. Electron donors can also form charge transfer complexes with electron acceptors.

Biological processes involve important redox (reduction-oxidation) reactions, biogenic energy is frequently stored and released by means of redox reactions as in photosynthesis that involves the reduction of carbon dioxide to form sugars and the oxidation of water into molecular oxygen. Most of the redox reactions in the environment are biologically mediated but may also occur abiotically. These reactions are influenced by biological activity as the availability of suitable reactants for electron transfer reactions are determined largely by such processes. Important abiotic oxidant/reductant couples include $Fe^{+2}/Fe(0)$, $S_{(s)}/HS^-$, and NO_3^-/NH_4^+. In geology, redox reactions are important to both the formation of minerals, mobilization of minerals, and in some depositional environments.

Reductants in organic chemistry are diverse. Electropositive elemental metals, such as lithium, sodium, magnesium, and iron, are good reducing agents. These metals donate electrons readily. So called hydride transfer agents, such as sodium borohydride and lithium aluminium hydride, are widely used in chemical synthesis, primarily in the reduction of carbonyl compounds to alcohols. It can also, as in this thesis, be used to reduce aromatic halides (Papers I and II). Another method of reduction involves the use of hydrogen gas with a palladium, platinum, or nickel catalyst. These are used primarily in the reduction of carbon-carbon double or triple bonds.

2.2.2 Microorganisms

Microorganisms play important roles in the global element cycles, such as the carbon- and nitrogen cycles, as well as in virtually all ecosystems where they act as decomposers of other organisms' dead remains and waste. There are, for instance, phototrophic and lithotrophic microorganisms, microorganisms in waters and anaerobic environments, and microorganisms that are able to grow not only under high temperatures but also at extremes of pH and salinity [65]. In short, in almost all parts of the biosphere where there is some liquid water there are microorganisms.

It is generally considered that biological reactions involving microorganisms are of major significance in determining the fate and persistence of organic compounds in aquatic and terrestrial ecosystems (c.f. above). Some microorganisms are involved in redox reactions in which they utilise a variety of metal-coenzymes. Porphyrins play important roles in many biological processes and iron porphyrins are a class of coenzymes involved in microbial redox reactions, other examples are transition metal coenzymes such as coenzyme F430 and vitamin B_{12} (containing nickel and cobalt, respectively) [66-68].

Research of the degradation of halogenated pollutants by microorganisms has shown that the main process is reductive dehalogenation [69-73], but also to some extent oxidative and fermentative dehalogenation [74].

The reductive dehalogenation under anaerobic conditions can be divided into abiotic, co-metabolic, and metabolic processes [74]. Metabolic dehalogenation is found in halorespiring bacteria whilst the co-metabolic processes are catalysed by metal iron-containing tetrapyrroles or co-enzymes, as described above [74].

Halogenated hydrocarbons of a wide variety are known to be dehalogenated by halorespiring microorganisms. The activity of a majority of halorespiring bacteria is inducible by halogenated substrates. For several halorespiring microorganisms with multiple dehalogenation activities, the respective enzymes are differentially expressed, depending on the substrate present [74]. Under anaerobic conditions, reactions such as methanogenesis, acetogenesis,

and sulfate reduction compete with the microbial dehalogenation. The redox potential of the terminal electron accepting process determines the ability of the microorganisms to compete for the hydrogen [75].

2.2.3 Paper I - Reductive debromination of PBDEs

For Paper I, a test of reductivity under controlled reaction conditions was developed, avoiding the multifactorial situation in the microbial methods. The reductive transformations presented in this thesis used sodium borohydride as the reductive agent, the background for this choice is described in detail in section 2.2.3.2. In Paper I, a set of 15 PBDEs with six to ten bromine substituents and varied substitution patterns were used as test compounds. During method development different reagents, such as lithium aluminium hydride and borane-tetrahydrofuran complexs, and reaction conditions were evaluated.

The reductive transformation of the PBDEs with sodium borohydride is a nucleophilic aromatic substitution (NAS) [76,77]. In general the results of the study indicate that the aromatic carbons of the diphenyl ether rings become more susceptible to NAS with an increasing number of bromine substituents. However, the study presented in Paper I show the BDE-181, substituted with seven bromines is distinctly more reactive than the other two heptaBDEs in the study (see Figure 2.1), even more reactive than some octaBDEs. This result indicates that the substitution pattern also influences the resistance to reductive degradation i.e. that congeners with one fully brominated ring such as BDE-181 are more prone to reductive transformation. This trend is confirmed by the results in Paper II where reduction of the fully brominated ring of the nonaBDEs was primarily debrominated. In the literature there are some studies of reductive debromination of individual PBDE congeners

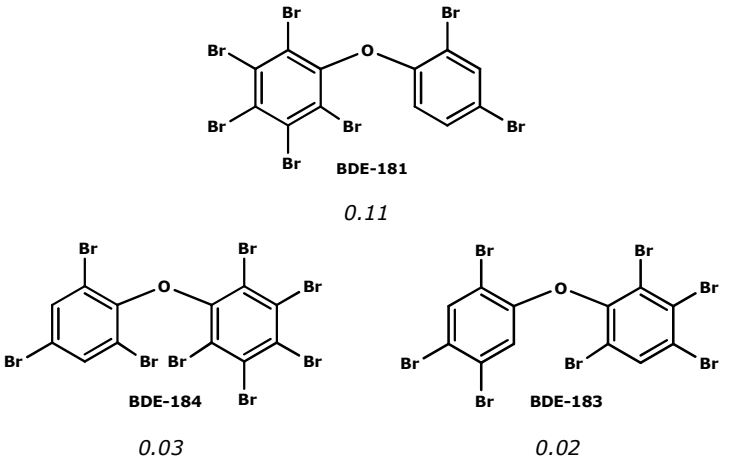

Figure 2.1. The heptaBDE test compounds BDE-181, BDE-183 and BDE-184. Italicised numbers indicate the relative reactivity of the compound as a fraction of that of BDE-209 which is set to 1.

[69,70,78-81]. Unfortunately, the specific reaction rates are seldom presented, and if so, few (if any) of the higher brominated DEs used in this thesis are included. Rewarding comparative discussions of relative rates is therefore not possible.

An important discussion regarding assessment of persistence is the issue of transformation products, as addressed elsewhere [82-85]. A chemical might in itself be quite rapidly transformed in the environment but its products might be highly persistent, e.g. DDT whose transformation product DDE is considerably more persistent than its precursor. To study transformation products was not an objective in Paper I. However, the method presented in Paper I yields, with some adjustments, opportunity for studying transformation products, as is done in Paper II. The availability of authentic PBDE reference standards is improving but e.g. the heptaBDEs are still only partly covered, and there is still analytical difficulties as coelutions of PBDE congeners to take into account. Hence, a complete identification of transformation products would involve a lot of, albeit qualified, guesswork. Admittedly, it would have been interesting to also look for potential formation of some of the congeners that are dominant in the environment, i.e. BDE-47, BDE-99, and BDE-100.

There is a multitude of highly diverse strains of microorganism in the environment. As described in Chapter 2.2.2, these species have different properties, even with the same general substrate preference. A study of microbial debromination of PBDEs by Robrock and co-workers (2008) confirms this [70]. The study investigated three dehalogenating cultures; a trichloroethylene enriched consortium containing multiple *Dehalococcoides* species and two pure cultures, *Dehalobacter restrictus* PER-K23 and *Desulfitobacterium hafnisense* PCP-1. All PBDE congeners were to some degree debrominated by the cultures. Most transformation products were common but in some cases produced in quite different concentration quotas and sometimes an additional congener was formed [70]. It has also been reported that the carbon source and carrier solvent added to a test culture can influence the formation of transformation products [86]. A study by He and co-workers (2006) show that the presence of PBDEs in a test culture acted inhibitory or toxic to the dechlorinating microorganisms present [73].

Robrock and co-workers (2008) studied microbial debromination of BDE-197, BDE-203, BDE-196, BDE-183, BDE-153, BDE-99, and BDE-47 [70]. They observed a comparatively lower debromination rate of the octaBDEs than the penta- and tetraBDEs tested, attributing this to the increased hydrophobicity, and thus increased adsorption to particles, that comes with increasing degree of bromination [70]. A basis for any assessment of chemical persistence is the acquiring of fundamental chemical properties of the test compound, such as vapour pressure and partitioning behaviour. The hydrophobicity of the highly brominated DEs makes them more likely to end

up in reducing environments but might also result in a high degree of adsorption to soil and sediment particles, removing them from the reducing microorganisms. By making use of the soil partition coefficient of the compound, the effective transformation rate in sediment can be calculated [80].

In order to follow the formation of lower brominated DEs in the environment, the preferred points of debromination can be assumed to be of relevance. There are many factors that influence the chance of a debromination at a specific position; a substituent in a sterically hindered position, a position that contributes to a lower state of energy for the compound, or in a position that is unaffected by a present enzyme will in all likelihood lower the reactivity of the compound. This will be discussed in more detail in Chapter 2.2.4.

The reductive debromination of PBDEs is not isolated to anaerobic environments but also occurs in photochemical reactions and reductive debromination has been investigated further by using e.g. zerovalent iron and electrolysis methods [78,79,87,88]. This will be discussed further in Chapter 2.2.4.

The method for determining relative reductivity that is presented in Paper I give the chemical reactivity of the test compounds in a strictly controlled environment. The reducing environment is easily acquired and no blanks need to be run to confirm that no other reactions take place. The determination of relative reaction rates makes assessment of the strength of the reducing environment unnecessary. Using a reductive agent of appropriate strength drastically shortens the reaction time as compared to most microbial methods [69,73,81,86,89]. As water solubility of halogenated organic pollutants is often limited, the possibility of using a relatively unpolar solvent affords total access to the test compound. The toxicity of the test compound is irrelevant to the process and no pre-adaptation needs to be considered. Sampling is simple and the concentration of substrate and reducing agent is environmentally relevant. The reactivities of the PBDE congeners tested in Paper II are presented in Table 2.1, as the half-life of BDE-209 divided by the test congener's half-life. This gives the fastest reacting compound, BDE-209, the reactivity 1 and the other test compounds reactivities as fractions of one.

Analysis

High Performance Liquid Chromatography (HPLC) was utilised for separation and analysis in Paper I and Paper IV. To use this technique when working with BFRs and other halogenated organic compounds is quite uncommon. However, the purpose of the analyses was to measure the disappearance of one specific compound, as opposed to the objective of comparative quantification (between PBDE congeners of different response for instance), and was found convenient and reliable for this purpose.

Table 2.1. Relative reductivity of the PBDE congeners testes in Paper I, presented in ascending relative reactivity.

Congener	Number of test reactions	Reactivity compared to that of BDE-209	Relative standard deviation (%)
BDE-209	10	1	12
BDE-208	10	0.35	11
BDE-206	10	0.34	14
BDE-207	10	0.33	14
BDE-198	10	0.23	21
BDE-203	10	0.21	22
BDE-204	10	0.13	25
BDE-181	10	0.11	30
BDE-201	10	0.054	11
BDE-202	10	0.030	19
BDE-184	10	0.028	25
BDE-196	10	0.024	23
BDE-183	10	0.023	23
BDE-153	5	n.r.o	n.r.o
BDE-154	5	n.r.o.	n.r.o.

n.r.o = no reaction observed

Solvent composition

Sodium borohydride is common reagent and fairly easy to handle. The solubility of sodium borohydride is unfortunately rather limited; it dissolves readily in water but by which it is also rapidly decomposed at neutral or acidic pH [90], and is soluble in alcohols but where it is also degraded. For the purpose of reducing a certain functional group in a synthesis, the fact that sodium borohydride can be degraded by the solvent used is of lesser importance. It may in fact be quite practical, but when it comes to creating a constant reducing condition in a reaction mixture it is unfortunate. Ethanol was eventually chosen as the reagent solvent. Sodium borohydride reacts slowly with ethanol at 20°C and is degraded but to a lesser degree than in water [90]. The sodium borohydride was added in large excess and the reaction time was kept to a minimum to ensure that the reaction conditions made it possible for the reaction to be of pseudo first order. It should be mentioned that the presence of a base would counteract the degradation but that would of course not give a purely reducing environment.

The PBDE test compounds, especially the highly brominated congeners, are more or less insoluble in ethanol because of their unpolar character. Tetrahydrofuran is a much more suitable solvent for the PBDEs and it was concluded that a mixture of solvents was needed. By using pure THF in an initial dilution step in the preparation of the test solutions, and a mixture of ethanol and THF (1:2) in the following step, a working solvent mixture produced.

Reaction temperature

As initial thought was to degrade test compounds of low reactivity at a, for practical purposes, satisfactory high rate, one could simply elevate reaction temperature. However, it was observed that a too high temperature accelerated the degradation of the sodium borohydride, often to a point where the data acquired during the experiments showed unacceptably low adherence to the straight line that confirms a pseudo first order reaction. Finally, the ambient temperature in the laboratory was chosen as reaction temperature, for practical reasons and because the rates of the reactions could still be kept within acceptable limits. An improvement of the method would be to find a more potent reduction agent that could reduce mono- to hexaBDEs, complementing the results in Paper I. By running hepta and octaBDEs already tested in the method presented in Paper I with this new reagent, a relationship between the two methods could probably be established. Hence, a scale of the reductivity of all levels of PBDE bromination could be constructed. It would furthermore be satisfying to determine the relative reaction rates of the heptaBDEs in a more strongly reducing environment.

Sensitivity to moisture

Like all hydrides, sodium borohydride is hygroscopic. The influence of water on the reactions was apparent during method development. Controlling the status of the solvents and the reagent to a satisfactory degree proved an impossible task. Drying agents for the solvents were evaluated but with unclear results. Inert atmosphere over the reaction mixture proved overly complicated from a practical point of view as the reaction produces hydrogen gas. The condition of the actual reagent was difficult to monitor, different suppliers gave reagents of apparently different strengths and batches of reagent from the same suppliers were of different quality. The latter was apparent because sometimes the product consisted of crystals and sometimes they had formed an aggregate, in all likelihood caused by water.

Finally the water issue was resolved by taking the following measures: all test compound solutions were prepared at the same time using freshly distilled THF and new absolute ethanol. The solutions were kept under helium. Reagent solutions were prepared from new sodium borohydride and new absolute ethanol. Finally, the differences between reaction conditions in separate sets was compensated by the use of a reference compound as described in the following section.

Reactivity control

Even though the assessment of chemical reactivity of potential pollutants is aimed to measure absolute reaction rates (reactivity constants) it is necessary to control the quality of the reactivity measurments carried out. Also, it is of

value to determine the relative reaction rates of chemicals being tested. Hence control reactions have been performed in parallel to the test reactions.

To detect and to give a possibility to correct for side effects from reagent or solvent status in a set of reactions, the results from test reactions were normalised by simultaneously running a control reaction. The latter reaction was always carried out using a reference compound and exactly the same reagent solution as for the test reactions, i.e. a control reaction. Instead of presenting reaction rates, relative reaction rates were calculated. Relating the test compound's reactivity to that of a reference compound in the corresponding controll reaction. This procedure was particularly important since it compensates for fluctuations in reaction temperature between different sets of reactions.

As examples, BDE-207 was used as a reference compound for the transformation experiments of BDE-209. BDE-209 was used as a reference for the experiments with the nonaBDEs. For the octaBDEs, BDE-207 was used as reference compound. BDE-203 was used as reference compound for the heptaBDEs, and BDE-181 was used in the testing of the hexaBDEs. The relative reactivities were finally recalculated to describe the reactivity of each individual test congener as a fraction of the reactivity of BDE-209. Accordingly, there are quite a bit of control to put in to be able to certify the quality of the measurments presented in Paper I.

2.2.4 Paper II – Reductive debromination of nonaBDEs

The fraction of nonabrominated diphenyl ethers released into the environment is quite small, it is present in the DecaBDE mixtures but only a couple of percent, as presented in Table 3.1. There is evidence that the nonaBDEs are increasing in the environment [91-94]. Environmental transformation of the technical DecaBDE and OctaBDE must be the source of changing congener patterns and formation of PBDEs not present in the technical PBDE mixtures.

The three nonaBDEs, BDE-206, BDE-207, and BDE-208, lack a bromine in the *ortho*-, *meta*-, and *para*-positions respectively (Figure 2.2). This enabled investigation of the preferred point of debromination in a reducing environment as the formed PBDE congeners indicate this.

The method for reduction of the three nonaBDEs was much the same as that presented in Paper I with only a few alterations. No reference reaction was used as the rate of reduction was considered of less importance; the focus of the study was to investigate the formed octaBDE products. Two time points, one and two hours, were however chosen to give some information of the kinetics of the reactions.

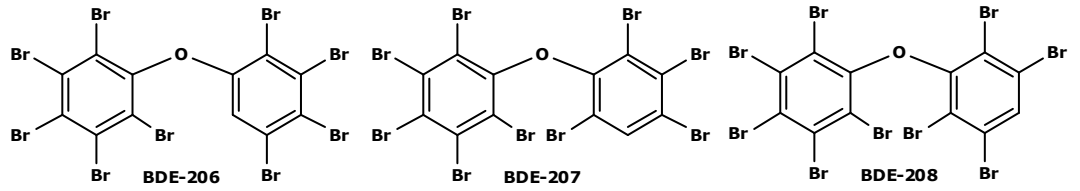

Figure 2.2. Structures of the three nonaBDE isomers, BDE-206, BDE-207, and BDE-208.

OctaBDE products

As presented in Paper II, the reductive debromination of the three nonaBDEs gave isomer-related transformation product patterns. Figure 2.3 shows the reductive debromination of BDE-206 and the distribution of transformation products. The test gave BDE-194, BDE-196, and BDE-199 as major products in approximately equal proportions. The reduction has taken place on the fully brominated ring of the nonaBDE, indiscriminately in *ortho*-, *meta*-, and *para*-position, respectively. The reductive debromination of BDE-207 (Figure 2.4) gave BDE-196 as major transformation product, and minor products were BDE-197 and BDE-201. The debromination forming these congeners took place on the fully brominated ring. Another minor product was BDE-203, where debromination had taken place on the already debrominated ring, in the *ortho*-position to the ether bond. BDE-208 (Figure 2.5) produced one major octaBDE congener, BDE-199, where debromination had taken place in the *ortho*-position of the fully brominated ring. BDE-201 and BDE-202 were minor products of debromination on the fully brominated ring in the *meta*- and *para*-positions, respectively. BDE-198 was formed as a minor product after debromination in the *ortho*-position of the already debrominated ring.

The results in Paper II indicate a preference for reductive debromination on the fully brominated ring, probably as a result of its more electropositive carbons. Further, the results point to a preference for debromination on the carbons in *ortho*-position to the ether bond. These positions are even more electron deficient than those merely affected by the fully brominated ring, as the electronegative oxygen of the ether bond will draw even more electron density from them.

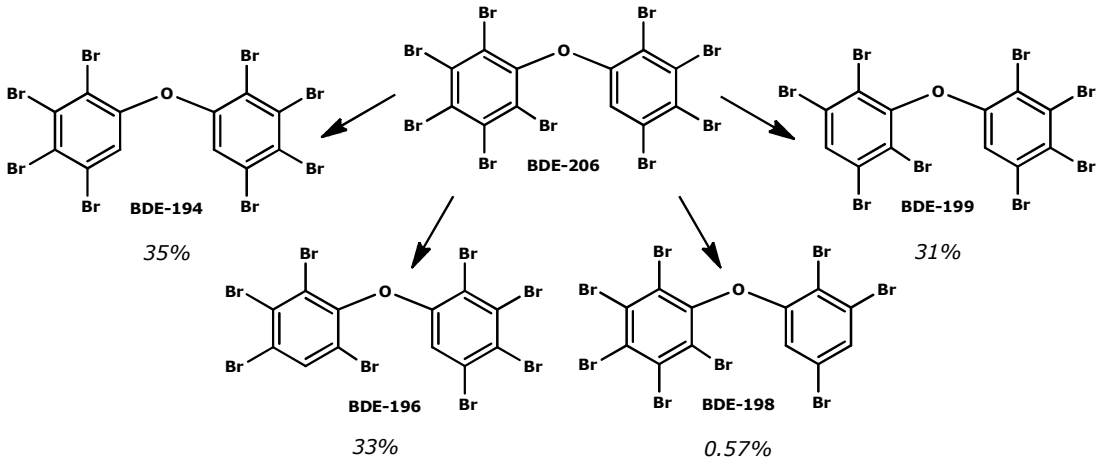

Figure 2.3. OctaBDE transformation products of reductive debromination of BDE-206 (2 hour sample). Percentages refer to amount of formed product related to the total amount of formed products.

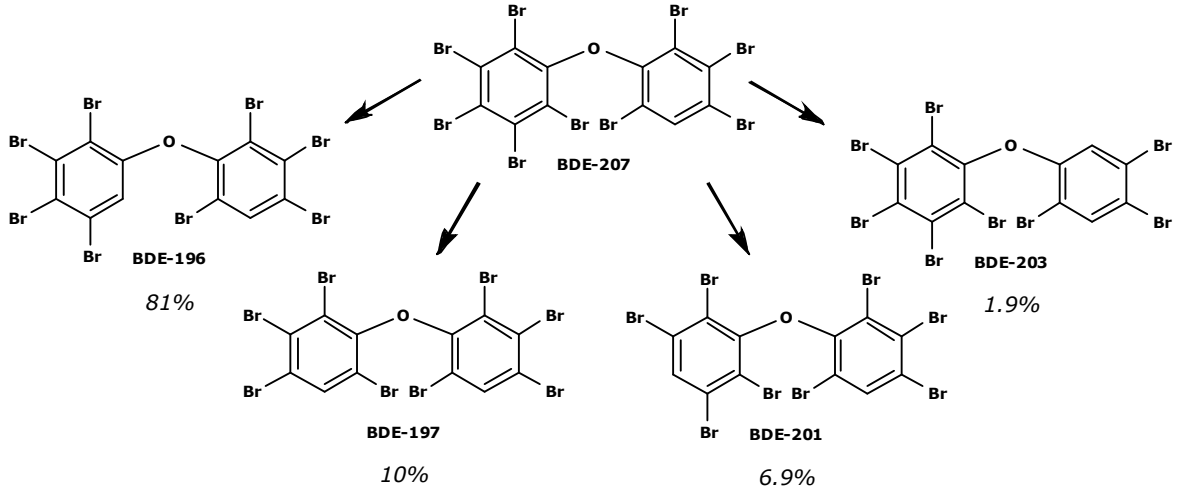

Figure 2.4. OctaBDE transformation products of reductive debromination of BDE-207 (2 hour sample). Percentages refer to amount of formed product related to the total amount of formed products.

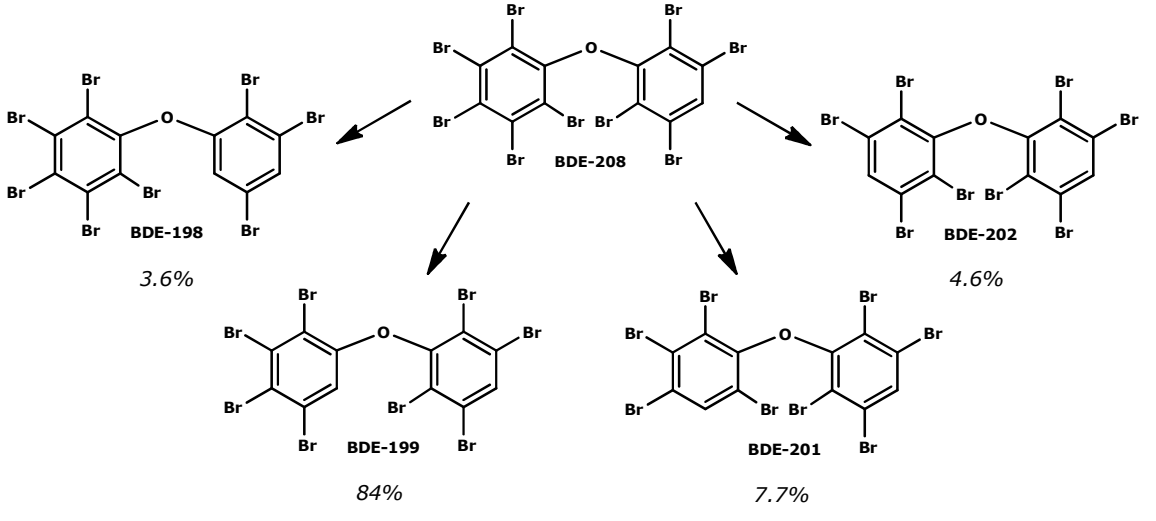

Figure 2.5. OctaBDE transformation products of reductive debromination of BDE-208 (2 hour sample). Percentages refer to amount of formed product related to the total amount of formed products.

The two time points give some confirmation of the results but it should be pointed out that it is possible that some octaBDE congeners are more rapidly formed than those identified as products, but are in turn quickly degraded. However, some confidence is put in the kinetic validity, based on the energetically favourable product formation.

As stated above, the results presented in Paper II indicate a preference for *ortho*-debromination of the nonaBDEs. In perspective; a study of reductive debromination by zerovalent iron has indicated a preference for *ortho*-debromination of tri- to pentaBDEs [78]. Tokarz and co-workers (2008) saw *ortho*- and *meta*-specific debromination of BDE-209 in a biomimetic system using vitamin B_{12} as reductive agent [80]. A study by Gerecke and co-workers (2005), demonstrating microbially mediated reductive debromination of BDE-209 for the first time, showed exclusive *meta*- and *para*-debromination [69]. Further, a study of microbial debromination of DecaBDE and OctaBDE technical mixtures has shown a preference for *meta*-debromination [73].

The image that emerges is that anaerobic microbial debromination generally does not lead to debromination of PBDEs in the *ortho*-position. One interpretation of this is that the energetically favourable debromination in the *ortho*-position may be sterically unavailable for microbial reactions.

It has been proposed that the origin of assumed debromination products from the technical PBDE mixtures in sediments and soils is phototransformations rather than microbial reactions [94]. Few studies of reductive debromination of the separate nonaBDE congeners, apart from the one presented in Paper II, have been conducted. A study of phototransformation pathways of the nonaBDEs has been presented by Davis and Stapleton (2009) [95], and a microbial debromination study by Gerecke and co-workers (2005) has looked at the nonaBDEs BDE-206 and BDE-207 [69]. Unfortunately, the two studies suffer from incomplete sets of octaBDE standards and satisfactory comparison is not possible. However, taking into account analytical difficulties, as described in "Analysis" below, and the lack of standards, there are similarities in outcome of the experiments. A re-analysis of the results from these studies, with complete octaBDE standards, will reveal just how similar.

Analysis

Polybrominated compounds, such as PBDEs and the novel BFRs mentioned below, are most commonly quantified using MS techniques operated in electron-capture negative ion mode (GC/ECNI-MS) [96-99]. ECNI is a relatively gentle ionisation technique. Another ionisation method utilised for BFRs is electron ionisation (GC/EI-MS) [100,101]. However, as GC/EI-MS requires highly purified samples its applicability when dealing with

environmental samples is sometimes limited, and because of its lower sensitivity it is usually used with high resolution MS (HR-MS).

GC/ECNI-MS using selected ion monitoring (SIM) is an advantageous combination when quantifying BFRs as the high abundance of the bromide ion isotopes (*m/z* 79 and *m/z* 81) give a high sensitivity of detection [101,102]. The ECNI spectra extracted from an analysis of a BFR depends on e.g. the instrument used, the type of reagent gas used, and reagent gas pressure. Another important factor is the ion source temperature. In general it can be said that using a high ion source temperature, the response decreases with increasing number of bromines [99].

For higher brominated PBDEs, the ECNI-MS technique also allows for the monitoring of separate brominated phenolate ions in SIM-mode, as utilised in Paper II, giving opportunity to distinguish between congeners with separate substitution patterns and fragmentation. A symmetrically substituted octaBDE will give tetrabromophenolate ions whilst an unsymmetrically substituted octaBDE gives tri- and pentabromophenolate ions. Cleavage of the PBDE ether linkage forms tri-, tetra-, and pentabrominated phenolate ions. The characteristic phenolate ions are most prominent from the congeners with eight or more bromines. ECNI normally almost exclusively gives the bromine ions $^{79}Br^-$ and $^{81}Br^-$ ions in the spectra.

Identification of co-eluting octaBDEs

Previous studies of octaBDE congeners by e.g. Davis and Stapleton (2009) and Gerecke et al. (2005) have only presented tentative identifications since not all congeners were available as standards at the time of the studies, and the separation of these isomers presents difficulties [69,95]. However, the identification and quantification of formed octaBDE transformation products presented in Paper II was conducted using a reference standard mixture containing all octaBDEs.

It is the congeners BDE-198, BDE-199, BDE-200, and BDE-203 that have presented difficulties in the identification of octaBDEs. In the studies mentioned above, one or other of these octaBDEs are presented as co-eluting congeners, but one or more of them were also absent from the standard used in the analysis.

As stated in "Analysis" above, in Paper II we used ECNI fragmentation and SIM for identification and quantification of the co-eluting congeners. The monitoring of the $^{79}Br^-$ and $^{81}Br^-$ ions as well as the tribromophenolate-, tetrabromophenolate- and pentabromophenolate fragment ions when analysing the separate congeners revealed unique fragmentation patterns. By optimising the chromatographic settings we found an elusion order of BDE-198, BDE-199, BDE-203, and BDE-200.

Evidence of environmental transformation of the PBDEs can be found in shifted relative proportions of congener levels in the environment compared to the technical mixtures. Further, verification of environmental transformations of PBDEs is the presence of congeners that were never in the products to begin with. A detailed analysis of the congener compositions of some of the most commonly used PentaBDE, OctaBDE and DecaBDE mixtures has been presented by La Guardia and co-workers [103]. Among octaBDE congeners that have not been identified in technical PBDE mixtures is BDE-205 which was found in the reductive transformation of BDE-206 (Paper II).

2.3 Hydrolysis-substitution-elimination reactions

Hydrolysis, elimination, and substitution (hse) reactions are of great importance to the transformation of chemicals in the environment. Looking at these reaction types from a perspective of creating a palpable set of test methods to assess chemical persistence, one has to look for the common denominators. These reaction mechanisms all involve a nucleophilic attack and we have therefore, within the scope of this project, developed one method for assessing the susceptibility of compounds to this type of transformation, denoting it hse.

The rate of an hse reaction is governed by aspects such as the strength of the nucleophile, hard-soft acid-base properties, steric hindrance, and the influence of the surrounding solvent. A set of diverse compounds reacted with nucleophiles of different properties will give different order of reactivities as the combination of reagent and target compound decides the rate of reaction. Water, a small and hard compound, is undoubtedly the most common nucleophile in our environment. However, there are also a number of naturally occurring soft bases, often containing sulfur, and bulky humic matter, which are also involved in hse transformations in the environment [68].

As previously addressed in this thesis, to develop a single method that encompasses all naturally occurring transformation reactions, hse reactions in this particular section, is not possible.

2.3.1 Paper III - Hydrolysis-substitution-elimination

The study of hse reactions in the context of Green and Bergman's attempt to develop a new strategy for persistency assessment was initiated by Rahm and co-workers in 2005 [104]. The reagent of the established method was sodium methoxide, which was dissolved in a mixture of methanol and dimethylformamide (DMF). The reactivity of the conditions was varied by

varying the solvent composition. The method developed by Rahm and co-workers was in Paper III subjected to evaluation to find aspects to further improve the methodology to achieve a more robust and possibly a simplified method. There was also a desire to test new compounds to add to those previously investigated.

The choice of sodium methoxide as the nucleophile for this study was made bearing in mind four dominant factors that affect reagent reactivity: nucleophilic strength, hard-soft acid-base interactions, steric effects, and solvent effects. The methoxide ion is a hard nucleophile, being small, strong, charged, and non-polarizable.

Hexachlorobenzene (HCB), pentachlorobenzene (PeCB), 1,2,4,5-tetrachloro-benzene (1,2,4,5-TeCB) and 1,2,4-trichlorobenzene (1,2,4-TCB) were selected as test compounds.

As expected, the susceptibility to undergo hse reactions was dependent on the number of chlorines on the ring, HCB gave the highest reaction rate and 1,2,4-TCB the lowest.

As it could be established that NAS was the only occurring reaction mechanisms second-order rate constant could be used to assess the relative susceptibility towards substitution.

Method development

The original nucleophilic substitution method (Rahm and co-workers, 2005) required the reagent solution to be produced not more than 14 days before use [104]. In Paper III the stability of the sodium methoxide solution was investigated. The results of these experiments indicated a stability of the reagent solution of at least 35 days after preparation, shown by the small variation of the pseudo first-order rate constants between experiments.

To ensure that the transformation of the test compounds was limited to the methoxide reaction, the method developed by Rahm and co-workers included purging the reaction solution with nitrogen. As the basis of Paper III was to improve and simplify the method, the possibility of removing this element was investigated. Our study found that the effect of exposure to air and possible moisture when the purging was removed and the sampling was conducted by opening the sealed arm of the flask, was very small. The more stable results without purging were considered an indication that this method was more accurate. Purging gave generally faster reaction rates and the dependency between reaction rate and solvent composition might perhaps allow the assumption that the purging gave methanol evaporation, hence resulting in a more active methoxide ion and faster reaction rates.

The strength of the methoxide ion is influenced by the surrounding solvent and its composition. When the methoxide ions are formed from methanol,

they are tightly associated to the solvent and the reactivity of the reagent is thereby hindered. The association is lessened by the introduction of a second solvent to the reagent solution, DMF, and the strength of the nucleophile is increased.

The relationship between reaction temperature and k_2, and between the MeOH:DMF ratio and k_2 were investigated using 1,2,4,5-TeCBz as test compound. Generally the experiments showed a decreased k_2 with an increasing part methanol in the solvent mixture. An elevation in reaction temperature gave an increase of the second order rate constant, e.g. a 10°C increase led to a k_2 increase of two to five times.

Transformation products

The study in Paper III also involved looking at the transformation products from the hse transformation of PeCB. A lack of appropriate reference standards gave no identifications of substitution patterns, but as no other products than methoxylated chlorobenzenes were found it could be concluded that the reaction was a pure nucleophilic aromatic substitution.

The reaction produced at least five methoxylated polychlorobenzenes.

2.4 Photolysis

The halogenated organic compounds addressed in this thesis, as well as a large number of other environmental contaminants, may undergo photochemical transformations when subjected to UV-light. These compounds contain delocalised π-electron systems which often enable absorption of photons, i.e. they have an energy gap between bonding/non-bonding, and anti-bonding orbitals corresponding to the energy of the photon. The absorbed energy can lead to photoluminescence, photosensitisation or photochemical reactions. The light that reaches earth is of wavelengths between 290 and 600 nm. The energy of such UV and visible light is of the same order of magnitude as that of covalent bonds that can thereby be cleaved as a consequence of light absorption.

Pathways and rates of photochemical transformations are commonly dependent on the type of solvent used, including e.g. its pH and ionic strength. Temperature has on the other hand little influence.

The ability of a compound to absorb light at certain wavelengths is described by its molar extinction coefficient, ε, which is an inherent property of the compound. Organic compounds may absorb light over a wide range of wavelengths with one or more absorption maxima. For quantification of photochemical processes the entire absorption spectra must be known.

The quantum yield of a photochemical reaction is the number of reactions occurring per absorbed photon at a specific wavelength:

$$\Phi_{ir}(\lambda) = \frac{total\ number\ of\ molecules\ transformed}{total\ number\ of\ photons\ (of\ wavelength\ \lambda)\ absorbed}$$

Reaction quantum yields have to be determined experimentally and can be used to estimate the overall transformation rate of a given compound.

2.4.1 Paper IV – Photolysis of some new BFRs

There are two general approaches to the study of photolytical transformation. The test compound, encased in a product or sorbed to a solid support of some sort, may be subjected to natural sunlight [95,105]. Or, the conditions of irradiation from natural sunlight can be constructed in a laboratory setting keeping the test compound dissolved in an appropriate solvent.

As a part of the persistency project this thesis is a part of, previously Eriksson et al has determined the photochemical degradation rates of a number of PBDEs by developing a method that utilises an artificial light source and a cylindrical reaction chamber where the test compound is kept in a solution [106]. Further, Bastos and co-workers have studied brominated phenols and hydroxylated PBDEs within the project [107]. In Paper IV the method developed by Eriksson was applied to some of the more recent BFRs; 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EH-TBB), Bis(2-ethylhexyl) tetrabromophthalate (BEH-TBP), 1,2-Bis(2,4,6-tribromo-phenoxy)ethane (BTBPE), and decabromodiphenyl ethane (DBDPE). The aim was to investigate the susceptibility of the test compounds to undergo photolytical transformation, quantum yields and product patterns.

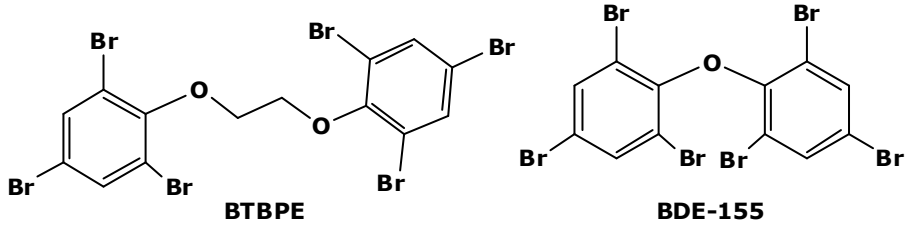

Figure 2.6. BTBPE and BDE-155.

BTBPE and BDE-155 have quite similar structures, as illustrated in Figure 2.6, and it was seen in the study (Paper IV) that their absorption spectra have similar characteristics. As discussed above, absorption above 295 nm is essential to make photolytical transformation important degradation pathways in the environment. Comparing the absorption spectra of BTBPE and BDE-155, they differ in that the two absorption maxima for BDE-155 are at higher wavelengths and have somewhat higher molar absorptivity (possibly

attributed to the inductive effect of the phenyl groups bridged by the ether oxygen). BTBPE absorps poorly at wavelengths above 295 nm and is much less reactive towards photolysis than BDE-155 and it can be assumed that phototransformation of BTBPE in the environment will be minor. BTBPE presents in an excellent manner the importance of absorpsion of UV light to induce photolysis.

The two compounds of Firemaster 550, BEH-TBP and EH-TBB, give similar absorption spectra but BEH-TBP shows much weaker absorption. The determined reaction rate constant of EH-TBB was almost twice that of BEH-TBP in the study. Results that require further investigation. The phototransformation studies of EH-TBB and BEH-TBP showed a clear indication that the products formed derive from reductive debromination, something which was also observed in similar studies by Davis and Stapelton [95]. When switching the solvent from pure methanol to a mixture of methanol and water (50:50), the half-life of the reaction became more than 2.5 times as long, no transformation products indicated reactions with water and the change in reaction rate is an indication that the only significant direct photolytic reaction was indeed reductive debromination.

Of the BFRs discussed in this thesis DBDPE is the most reluctant to be solubilised in any solvet, something which causes great difficulties in transformation studies such as those conducted in Paper IV. Instead of methanol, isopropanol was chosen as solvent as DBDPE is slightly more soluble in this solvent. As indicated in the comparison of BTBPE and BDE-155, the effect of the phenoxy-substituent on the absorption of a PBDE is important. The absorption spectra of DBDPE and BDE-209 illustrate this as BDE-209 shows higher absorption in the wavelength area of interest. Previous studies of BDE-209, by the same method as in Paper IV, give that the rate constant for the investigated phototransformation is almost five times higher than that of DBDPE. In all studies of phototransformation in Paper IV, only reductive debrominated was observed, except for in the investigation of DBDPE. This minor product is interpreted to be a substitution of a bromine with a methyl group instead of a hydrogen, a reaction not unlikely when using isopropanol as solvent.

Based on the physicochemical properties of the novel BFRs tested here, discussed in some detail in Chapter 3.3, it is reasonable to hypothesize that most of these compounds are bound to surfaces of particles (cf. log K_{OC}). Accordingly the compounds are, in part, shielded from undergoing photolysis. An example of this is the result reported of unexpectedly high concentrations of BDE-209 on window glass as assessed in Toronto [108].

2.5 Chemical reactivity as a tool for estimating persistence

Environmental modelling is an increasingly dependable support in legislative decisions concerning risk assessment of chemicals, originating in unprecedented computational power and scientific understanding of environmental processes. The overwhelming complexity of our environment, e.g. the geographical and seasonal span of temperatures and precipitation, indicate the difficulties in constructing models that will provide reliable answers to our questions. A model needs a deliberate level of generalisation to arrive at a manageable system that does not grossly over-empathise or underestimate the factors that a pollutant will encounter in the environmental compartments.

In the persistence assessment system proposed by Green and Bergman, five types of reactions: oxidation, reduction, photolysis, hse, and radical reactions, are considered to encompass all possible transformation reactions. To date, all but radical reactions have been investigated [104,109-113]. The reactivity of a number of known environmental pollutants has been determined. Further, the system suggests the creation of an environmental matrix that describes the likelihood of the five reaction types to occur, each type within each compartment. This means that in a quite simple manner, the complexity of possible transformations in one and the same compartment can be included in the model.

The substance reactivity, as determined in the test reactions, can then be multiplied with the environmental matrix affording the compartmental transformation rates of the test chemical.

A key to a successful model will be the creation of the environmental matrix. A collaboration of experts using all information available will be necessary to arrive at an adequate model. There are other factors in chemical assessment that cannot be determined in a lab either, but requires the evaluation of experts. Distribution of a chemical in the environment can largely be based on its physico-chemical properties, e.g. log K_{oc} and log K_{ow}, but the exact effects of these properties on chemical transformation is less straight forward. If a compound will adsorb tightly to particles, to what extent will it be available for reactions? Are bound residues to be considered removed from the environment? How can the environmental pH be accounted for when determining the partitioning behaviour of a polar compound?

The test chemicals used so far in the method development have been quite similar regarding chemical structure and properties. This has been beneficial in discussions concerning the effects of small structural differences on the overall reactivity of a compound. However, for the continued development of the project a wider set of reagents and conditions, as well as test compounds, need to be involved. This is not a weakness as the testing of chemical reactivity under controlled conditions allows for the use of standards.

Standard compounds can be used to determine relative reactivities within one and the same method but also as a reference point between different methods.

To arrive at dependable assessments of persistence there is a chain of factors that need to fit together. These are data of the physicochemical properties of the compound, the choice of tests used to determine its degradability, the model of the environment the test results are applied in, and finally and most importantly, how we interpret the results. We believe that tests performed under controlled repeatable conditions are essential to be able to perform accurate assessments of chemical persistence.

3 Test chemicals

The present thesis has primarily concentratied on chemical reactivity of BFRs but for methodological reasons also some other compounds are included. The brief presentations of the test compounds below is aimed to give an introduction to the chemicals with references to literature where comprehensive data can be searched for each of the compounds.

3.1 PBDEs

Polybrominated diphenyl ethers have been widely used as flame retardants in a variety of consumer products and electronics [28]. A general structure of PBDEs is shown in Figure 3.1. There are 209 possible PBDE congeners of varying substitution pattern and degree of bromination, numbered in accordance with the system for PCB nomenclature established by Ballschmiter et al (1980) [114] and further developed [115].

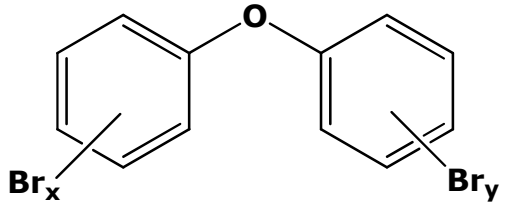

Figure 3.1. A general structure of PBDEs, x = 0-5, y = 1-5.

The PBDEs have been or are produced as three types of technical products; PentaBDE, OctaBDE and DecaBDE [28]. A general congener composition of the products is presented in Table 3.1. Approximately 66,000 metric tons of PBDEs were used worldwide in 2001 but due to the legislative and voluntary restrictions since 2004 the production has decreased [28].

Table 3.1. A general PBDE homologue group composition of commercial mixtures [116].

	DecaBDE mixture	OctaBDE mixture	PentaBDE mixture
DecaBDE	> 97%	< 1%	
NonaBDEs	< 3%	10–11%	
OctaBDEs		31–35%	
HeptaBDEs		44%	
HexaBDEs		10–12%	4–8%
PentaBDEs			40–60%
TetraBDEs			24–38%
TriBDEs			
DiBDEs			
MonoBDEs			

The very first report of PBDEs in biota was made in fish samples from the Swedish river, Viskan, in 1981 [117]. A time trend study of organohalogen contaminants in breast milk by Norén and Meironyte in 2000 revealed increasing concentrations of PBDEs in the milk [118] drastically changing the interest of these pollutants among scientists in the environmental field. Several studies have confirmed this trend [118-122] but during the last decade a decreasing trend of most of the PBDE congeners have been shown [123]. PBDEs have, by now, been detected in basically all environmental matrices and all over the world, including the Arctic [124].

Technical PentaBDE and OctaBDE mixtures were banned in the European Union in 2004 [125] and was concurrently phased out by the only US producer [126]. The use of DecaBDE in applications in electric and electronic equipments and products was restricted from July 1st 2008 according to the EU RoHS directive [127]. A phase-out of DecaBDE production in the US has been set to 2012 whilst the use of DecaBDE has no restrictions in Asia [128].

Table 3.2. Physico-chemical properties of PBDE homologue groups as predicted from Quantitative Structure Property Relationships (QSPR), adapted from Wania et al, 2003 [129]. (P = vapour pressure, S = water solubility, K_{OA} = octanol air partitioning coeffient, K_{OW} = octanol water partitioning coeffient, K_{AW} = air water partitioning coeffient.

	Log P_L	Log S_L	Log K_{OA}	Log K_{OW}	Log K_{AW}
MonoBDEs	−1.27	−2.12	7.81	5.08	−2.51
HeptaBDEs	−5.96	−5.06	12.78	7.49	−4.22
OctaBDEs	−6.74	−5.55	13.61	7.90	−4.50
NonaBDEs	−7.52	−6.04	14.44	8.30	−4.79
DecaBDE	−8.30	−6.53	15.27	8.70	−5.07

The properties of the PBDEs vary with degree and pattern of bromination. The vapour pressure and water solubility of the PBDEs can be expected to decrease with each additional bromine substituent whilst the partitioning into

fatty tissue can be expected to increase. An overview of estimated physico-chemical properties of some PBDE homologue groups is presented in Table 3.2.

Studies indicate that the PentaBDE of the three commercial PBDE mixtures cause adverse effects at the lowest doses [28,29]. The congeners in these mixtures are relatively small and thereby more readily bioavailable than the highly brominated DEs and have further been shown to bioaccumulate [130-133]. The adverse effects of the PentaBDE mixtures include altered reproductive function; neurodevelopmental effects; foetal toxicity and teratogenicity, perturbed thyroid homeostasis; irreversible motor and behavioural effects that deteriorate with age; immunotoxicity; deformed thyroid, liver, and kidney [27-29,134]. The DecaBDE has been exempted from regulation as the reported levels in biota were low, attributed to its low bioavailability. It is persistent; sediments and soils were considered more or less permanent sinks and transformation of the mixture into lower brominated DEs was considered negligible [135]. As previously discussed in this thesis, a number of studies have identified naturally occurring debromination transformations indicating that the DecaBDE mixture is a source of lower brominated DEs. Further, metabolism of BDE-209 has been observed in several studies of e.g. fish, birds, and humans [130,136-138].

3.2 Chlorobenzenes

There are twelve possible chlorinated benzene (CBz) compounds; the general structure is shown in Figure 3.2. The mono, di, and trichlorobenzenes had important industrial applications as solvents and pesticides, and triCBz were used as heat transfer agents together with PCBs as well. Mixtures of tri and tetraCBz were used to control shellfish predators [139]. Hexachlorobenzene (HCB) was used as a fungicide and an additive to rubber products [139].

CBz are released into the environment during manufacturing or use as intermediates in production of pesticides and other chemicals. As CBz can be produced in incomplete combustion they are also released from incinerators and hazardous waste sites [140]

Many PCBz are ubiquitous environmental pollutants. Both HCB and Pentachlorobenzene (PentaCBz) are, as mentioned is Chapter 1.1.2, regulated under the Stockholm Convention [9].

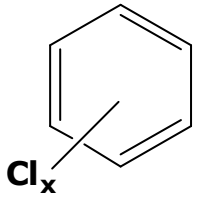

Figure 3.2. A general structure of chlorobenzenes, x = 1-6.

The higher chlorinated benzenes bioaccumulate and bioconcentrate [140,141], and acute and chronic toxicity generally increases with the level of chlorination. All PCBz are lipophilic, increasingly so with degree of chlorination. In general, a positive correlation exists between partition coefficient and degree of bioaccumulation.

HCB has adverse effects on organ development, the liver, and reproduction. It is known to cause porphyria cutanea tarda [141] and it is anticipated to be a human carcinogen [141].

The physicochemical properties of CBz suggest that released into the environment are likely to be volatilised to the atmosphere. The Henry's law constants measured for CBz suggests that they are readily volatilised, especially from aquatic systems with long residence times, such as large lakes and oceans. However, CBz released to water may also be adsorbed onto sediment, especially if it is rich in organic matter. CBz may also adsorb to soils of similar characteristics but volatilisation from soil is also likely.

In general, the solubility of chlorobenzenes in water is low (decreasing with increasing chlorination), flammability is low, the octanol/water partition coefficients are moderate to high (increasing with increasing chlorination), and vapour pressures are low to moderate (decreasing with increasing chlorination) [140].

3.3 Novel BFRs

The restrictions of brominated flame retardants, such as the PBDEs, have lead to a development of alternatives. Within the scope of this thesis, four novel BFRs have been investigated; BTBPE, EH-TBB, BEH-TBP, and DBDPE. Their structures can be seen in Figure 3.3 and these chemicals are described closer below. Some other BFRs, still to be found in the technosphere, include for instance HBCDD, and tetrabromobisphenol A bis(2,3-dibromopropyl ether) (TBBPA-dbpe). Like the PBDEs, these compounds are seeping out into our environment and as they are continually produced, it follows that their potential for transformation is of interest.

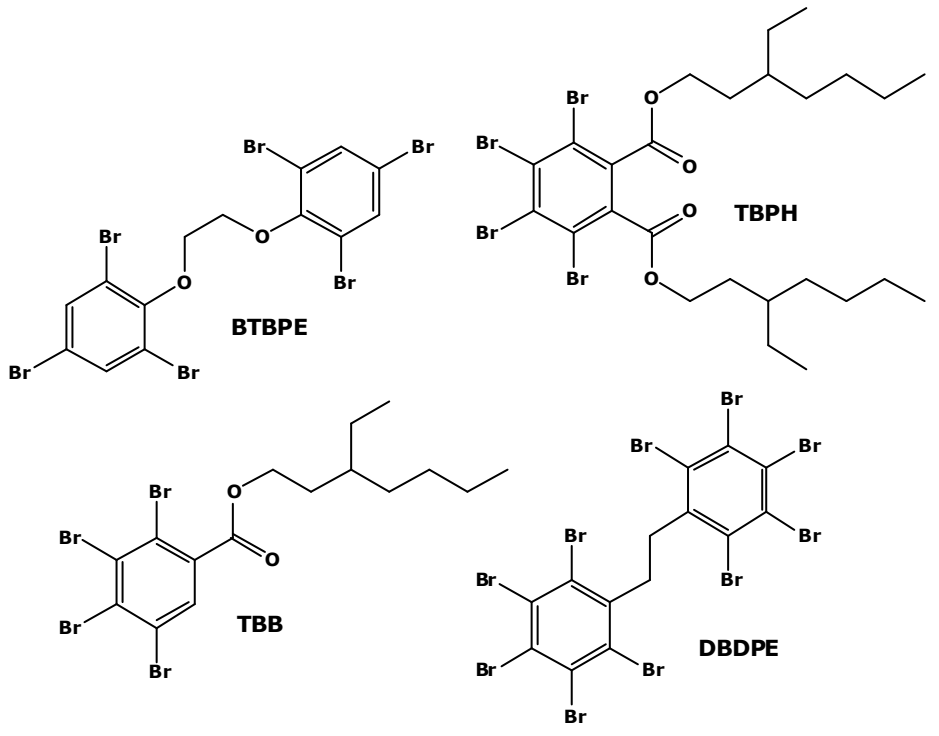

Figure 3.3. Structures of the novel BFRs: BTBPE, TBPH, TBB and DBDPE.

3.3.1 BTBPE

As a replacement for the banned OctaBDE products, BTBPE was put on the market as FM-680 by Great Lakes Chemical, now a part of Chemtura Inc [142]. BTBPE is used in production of plastic materials that require high manufacturing temperatures and light stability e.g. thermoplastics, high impact polystyrene, and acrylonitrile butadiene styrene terpolymer [143]. In 2001, worldwide production/usage was estimated to 16,710 tons [144].

A study by Wu and co-workers (2011) suggests that BTBPE is highly bioaccumulative and may have potentially high environmental risk [145]. BTBPE has a log K_{ow} of 8.3±0.7 (determined from calculated properties, SciFinder), which indicates a lipophilic character. The size of BTBPE makes its absorption over the gastrointestinal tract quite limited; a study of metabolisation and fate of BTBPE in rats showed > 94% excretion of the chemical [143]. This compound has a low vapour pressure, $3.9*10^{-10}$ Pa, and a log K_{oc} of 5.7 (determined from calculated properties, SciFinder) suggesting that it will adsorb to particles and is likely to partition to soils and sediments. Stapleton and co-workers reported BTBPE in house dust [146], and it was first time detected in air from electronics recycling plants [147,148], and in bird eggs [149].

In studies, BTBPE has shown weak acute toxicity [150], a slight porfyrinogenic effect [151], and no genotoxicity [152].

Combustion of BTBPE mainly produces 2,4,6-tribromophenol (TBP), which is neurotoxic [153], and 2,4,6-tribromophenyl vinyl ether [154]. Minor products of interest are polybrominated phenoxyphenols and dibenzo-*p*-

dioxins [154]. BTBPE has further been shown to produce hydroxylated metabolites [143].

3.3.2 EH-TBB and BEH-TBP

Two novel flame retardant components are EH-TBB and BEH-TBP. These are used together in the fire retarding products Firemaster 550 (FM 550) and BZ-54, and BEH-TBP alone in DP-45 (Great Lakes Chemical, Chemtura Inc). Both compounds are low production volume (LPV) chemicals within the EU; 10 to 1,000 tonnes per importer/producer are put on the market each year [155]. Little is known of their toxicity and potential for bioaccumulation.

The log K_{ow} of EH-TBB and BEH-TBP are 7.7±0.8, and 9.3±0.7 (determined from calculated properties, SciFinder). Further, the log K_{oc}s are 5.6 and 6.5 (SciFinder), respectively. These data, in combination with a very low vapour pressure, $3.7*10^{-7}$ Pa for EH-TBB and $1.5*10^{-11}$ Pa for BEH-TBP (SciFinder). indicates strong potential for partitioning into lipids, little partitioning to the air compartment, and tight binding to particles.

A study on house dust found both of the compounds in FM550 and the ratio of EH-TBB and BEH-TBP varied between samples and deviated from that observed in the original product [146]. Further, these novel BFRs have recently been detected in dolphins and porpoises from southern China. The levels of BEH-TBP were comparable to that of ΣHBCDs in some porpoise samples [156].

3.3.3 DBDPE

DBDPE is by the industry considered a suitable alternative to the DecaBDE commercial mixtures. It has been used as an additive in e.g. high impact polystyrene (HIPS) and in wire and cable coatings since the beginning of the 1990s [157]. Because of its high molecular weight it was believed, as BDE-209, not to be bioavailable, which has proved to be incorrect [130,138,158,159].

As the other novel BFRs discussed here, DBDPE also has a low vapour pressure, $2.9*10^{-15}$ Pa (SciFinder), a high log K_{ow}, 11.7±0.9 (SciFinder), and a high log K_{oc} of 7.0. Hence, it is also most likely to be found adsorbed to particles and/or in soils and sediments. Despite its size, it can be taken up via e.g. gastric membranes, where it will partition into fatty tissue [91,131,160].

There are few studies of DBDPE in the environment. Kierkegaard et al. (2004) was the first to detect the flame retardant in sewage sludge, sediment, and indoor air from Europe [161]. Studies have shown its occurrence in house dust [146], it has further been found in samples from 2002 and 2005 in sewage sludge from a wastewater treatment plant [162], and has been detected in the aquatic food web [131]. Bioconcentration of DBDPE was not detected

in a study on Japanese carp [163], but biomagnification between trophic levels has been observed [131].

The toxicity of DBDPE to mammals has been reported as very low [164], and this because of its poor bioavailability. Nakari and Huhtala (2010) have studied its effects on water fleas (*Daphnia magna*) finding the compound bioavailable despite its high molecular weight. Their investigations on water fleas and zebrafish (*Danio rerio*) further showed effects on hepatocyte detoxification metabolism and esterogenic properties of the compound [165].

4 Concluding remarks

The work presented in this thesis adds valuable material to the project for the development of a new method for assessment of persistence. A few major conclusions are extracted and presented below. These are:

A robust method for assessment of reductions of environmentally relevant brominated compounds have been developed but further work need to be applied to find alternative methods for reductive reactions in general (Paper I and II).

The involvement of microorganisms and biological matrices in persistence assessment appear to cause poor reproducibility and limited applicability. If a combination of the robust abiotic method for determination of reductivity presented in Paper I and II, and the physicochemical properties of the test compounds, will give results that mirror environmental observations remains to be investigated.

The evaluation of the previously developed method for determination of susceptibility to hse reactions gave satisfactory results as the method could be further simplified (Paper III).

The photolytical transformation study of novel BFRs presented in this thesis show how structurally related compounds often bear similar reactivity traits (Paper IV). The novel BFRs investigated are quite alike the “old” ones and further testing will show if these will go unrestricted.

Finally, to arrive at a point where the proposed assessment method can be properly tested, further work is still needed. A method for determination of susceptiblity to radical reactions needs to be developed, or possibly adopted based on the work done by others. It is also likely that the assessment methods previously developed require evaluation and further development. The assessment of persistence using determination of chemical reactivity as base will require detailed knowledge of the other properties of the compound and how these will affect the strength of environmental transformation it will face.

The final stage of the "persistency" project development will be the construction of the mathematical matrix describing the environment and the combination with reactivity data and physicochemical properties affording a persistence assessment.

5 Svensk sammanfattning

I det lagstadgade skyddet av vår miljö och hälsa är bedömningen av kemisk persistens en viktig del. Av det enorma antal kemikalier som finns på marknaden idag så har få genomgått en grundlig riskbedömning. Det finns ingen genomgående koordinering mellan olika nationella och internationella riktlinjer för persistensbedömning och metoder för bedömning av bionedbrytning uppvisar låg reproducerbarhet som en följd av dess komplexa natur.

2005 presenterade Green och Bergman ett förslag till en ny sorts bedömning av kemikaliers persistens. Genom att bestämma testsubstansernas kemiska reaktivitet i kontrollerade abiotiska laboratorieförsök kan reproducerbarheten, och att nedbrytningshastigheten bara reflekterar den tilltänkta reaktionstypens omvandling av testsubstansen, säkras. Substanserna testas i metoder för miljömässigt relevanta omvandlings- och nedbrytningsreaktioner, reduktion, oxidation, hydrolys-substitution-elimination (hse), radikalreaktioner, och fotolys. Den bestämda kemiska reaktiviteten, och data om substansens fysikokemiska egenskaper, läggs sedan till en matematisk matris som beskriver potentialen för de olika nedbrytningsreaktionerna i luft, vatten, jord och sediment. Detta ger förutsättningar för en bedömning av testsubstansens persistens.

Testmetoder för oxidation, fotolys och hse reaktioner har tidigare utvecklats inom Green och Bergmans persistensprojekt. I den här avhandlingen har en testmetod för reduktion av relevanta kemikalier utvecklats och har vidare använts för att bestämma nedbrytningsprodukter från reduktiv debromering av de tre nonabromerade difenyletrarna. Metoden har vidare använts för studier av 15 olika polybromerade difenyletrar. Metoden för bestämning av hse som utvecklats tidigare, har utvärderats och vidareutvecklats i en studie med utvalda klorbensener. Fyra bromerade flamskyddsmedel har undersökts med fotolysmetoden som etablerats inom projektet, omvandlingsprodukter studerades och kvantutbyten bestämdes. Alla studier som presenteras i den här avhandlingen är avsedda att utveckla och bygga på projektet för utvecklandet av en ny metod för bedömning av persistens. De presenterade resultaten bidrar även med viktig information om egenskaper och omvandling av några vanliga halogenerade organiska miljöföroreningar.

6 Acknowledgement

Ja, det blir ett långt ”tacktal”, orkestern kommer hinna börja spela innan det är färdigläst, men det är ju så många jag vill tacka! Många utropstecken blir det också...

Åke – Jag tackar ödmjukast för förtroendet. Jag lämnar institutionen med ovärderlig kunskap och unika erfarenheter, allt tack vare dig!

Johan – Min härliga biträdande handledare! Hur det går? Det går som vanligt; alldeles utmärkt! Tack för allt!

Maria, Per, Göran – Mina adopterade biträdande handledare under min avsaknad av sådan. Tack för allt! Maria, för en ovärderlig insats som fick allt att äntligen börja rulla. Per, för rätt ord vid rätt tillfälle och optimism, diskussioner och pepp! Göran, för din tajming, pratsamhet, välbehövliga organkemikunskap och humor. Tur att du inte är bitter ;-).

Ioannis – Tack för all hjälp och allt du lärt mig, tack dessutom för intressanta diskussioner om världen där ute!

Anita – Tack för all hjälp, omtanke och peppning!

Margareta, Lillemor, Lotta & Birgit – Tack för hjälp och stöd!

Tati – Underbara, briljanta du! Jag hade obeskrivlig tur när jag hamnade på samma lab som dig på Allmän kemi hösten -99 och ännu mer så när vi kunde bli arbetskamrater och rumskompisar här på miljökemin. Tack för allt! ♥

Ronnie – Illegitimi non carborundum! Thanks for everything mate ☺!

Yanling – I’m so happy that you came to the lab and it was a pleasure working with you!

Maria S – En av mina bästa studenter blev en av de bästa kollegorna! **Andreas** – Tur att vi slapp Pincer-komplexen och tog oss hit istället ☺! Tack för alla uppriktiga och roliga pratstunder, för att inte tala om en briljant zombiefest!

Johan F – Låt mig säga såhär: Må baconet flöda, må dina gröna bönor alltid vara krispiga, må mandelmassan lysa med sin frånvaro. Må dina gubbar alltid besegra sina motståndare och din klan likaså. Live long and prosper my friend. ♥

Linda – Ingen är som du, fortsätt följa ditt hjärta och din vilja! **Anna S** – Vänliga, varma Västeråsaren! **Karin L** – Du lyckas vara varm, snäll och kompromisslöst rak samtidigt; otroligt ovanligt! **Jessica** – Kul att du kom tillbaka och bra att vi bägge skaffat bättre smak!

Hans – Tack för alla pratstunder, insikter och uppriktigheter! **Hitesh** – Best of luck with your post doc and, I’m expecting, future brilliant academic career! Dags att lära dig lite mer svenska nu kanske ;-)? **Emelie** – Du kommer

också ta dig i mål, och aldrig få slut på något gott till fikat hoppas jag ;-)! **Anna V** – Tack för alla bittra och mer eller mindre konstruktiva diskussioner. Du har ett av mina absoluta favoritskratt, jag blir på gott humör bara av att tänka på det!

Cecilia och **Anna-Karin** – Mina gamla studenter ☺! Jag önskar er all lycka med doktorerandet och en strålande framtid! **Yin** – I wish you success in your research and a good time here in Sweden with lots of delicious desserts ;-)!

My deepest thanks to **Sharam** for the auto-injector (aka **R2D2 II**) you lent me. Without it I never would have been able to make it!

Varmt tack till **alla miljökemister** som passerat genom institutionen genom åren; **Hrönn**, **Sara**, **lill-Anna**, **Daniel**, **Ulrika**, **Anna M**, och **Johanna** för att nämna några, det har varit både roligt och lärorikt att vistas bland er!

¤ Daphne, Kajsa och Pirkko ¤

♥ Tack till mina kära vänner; nya, gamla, och ny-gamla. Några specifika måste nämnas: Älskade **Maria U**; thailunch och efterföljande pratpromenad är välgörande för själen. **Erik**, tack för alla kravlösa söndagspromenader. Finaste **Helena**, tack för att du finns där, trots att där rent geografiskt är en aning långt borta fortfarande. Herzlichen Dank **Astrid**! Merci à **Sophie**, je n'ai pas dû devenir une pirate ;-)!

♥ Familjen **Erixon** för att ni släppte in mig i er gemenskap precis som jag var och är!

♥ Min älskade familj; **Ninni**, **Pelle**, **Nisse med familj**! Jag vet vilken tur jag har som har en så kärleksfull, rolig och stöttande familj! Extra tack till mamma för all sparring, hjälp och inspiration!

♥ Min älskade **Jonas**. Den vackraste stunden i livet var den när det blev vi, och alla stunder med dig sedan dess. Tack för att jag får vara din!

7 References

1. Cefic: World chemical sales in 2009. (2011) http://www.cefic.org/Facts-and-Figures/Profile-of-the-Chemical-Industry/World-Chemical-Sales-by-geographic-breakdown/

2. Turusov V., Rakitsky V., Tomatis L. (2002). Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risks. *Environmental Health Perspectives*, 110, 125-128.

3. WHO (1979): Environmental Helth Criteria 9. DDT and its derivatives. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

4. Ratcliffe D.A. (1967). Decrease in eggshell weight in certain birds of prey. *Nature, London*, 215, 208-210.

5. Helander B., Olsson A., Bignert A., Asplund L., Litzén K. (2002). The role of DDE, PCB, Coplanar PCB and eggshell parameters for reproduction in the white-tailed Sea Eagle (*Haliaeetus albicilla*) in Sweden. *Ambio*, 31, 386-403.

6. Rogan W.J., Chen A.M. (2005). Health risks and benefits of bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT). *Lancet*, 366, 763-773.

7. Beard J. (2006). DDT and human health. *Science of the Total Environment*, 355, 78-89.

8. van den B.H. (2009). Global status of DDT and its alternatives for use in vector control to prevent disease. *Environmental Health Perspectives*, 117, 1656-1663.

9. Stockholm Convention: Stockholm Convention on persistent organic pollutants (POPs) 243. (2009) www.chm.pops.int/

10. WHO: WHO gives indoor use of DDT a clean bill of health for controlling malaria. (2011) http://www.who.int/mediacentre/news/releases/2006/pr50/en/

11. Rahman, MM (2006). Uses of persistent organic pollutants (POPs) in Bangladesh Department of environment and forests, Government of Bangladesh, Bangladesh, 56 .

12. Rahman, MM (2006). Survey and research on DDT and PCBs in food items and environmental samples Department of Environment and Forests, Government of Bangladesh.

13. Wong M.H., Leung A.O.W., Chan J.K.Y., Choi M.P.K. (2005). A review on the usage of POP pesticides in China, with emphasis on DDT loadings in human milk. *Chemosphere*, 60, 740-752.

14. Chakraborty P., Zhang G., Li J., Xu Y., Liu X., Tanabe S., Jones K.C. (2010). Selected Organochlorine Pesticides in the Atmosphere of Major

Indian Cities: Levels, Regional versus Local Variations, and Sources. *Environmental Science and Technology*, 44, 8038-8043.

15. Erickson MD (2001): Introduction: PCB properties, uses, occurrence, and regulatory history. Robertson LW, Hansen LG (eds) In: PCBs. Recent advances in environmental toxicology and health effects. The University Pess of Kentucky, Kentucky, USA, p xi-xxx.

16. Drinker C.K., Warren M.F., Bennett G.A. (1937). The problem of possible systemic effects from certain chlorinated hydrocarbons. *Journal of Industrial Hygiene and Toxicology*, 19, 283-311.

17. Koppe, J, Keys, J (2001). Late lessons from early warnings: the precautionary principle 1896–2000 22 European Environment Agency, 64-75.

18. Jensen S. (1966). Klorerade bifenyler i naturen. *Nordforsk, Biocid-information.*,

19. Jensen S., Johnels A.G., Olsson M., Otterlind G. (1969). DDT and PCB in marine animals from Swedish waters. *Nature*, 224, 247-250.

20. Aoki Y. (2001). Polychlorinated Biphenyls, Polychlorinated Dibenzo-p-dioxins, and Polychlorinated Dibenzofurans as Endocrine Disrupters-What We Have Learned from Yusho Disease. *Environmental Research*, 86, 2-11.

21. Larsen J.C. (2006). Risk assessments of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in food. *Molecular Nutrition & Food Research*, 50, 885-896.

22. Schantz S.L., Gardiner J.C., Aguiar A., Tang X., Gasior D.M., Sweeney A.M., Peck J.D., Gillard D., Kostyniak P.J. (2010). Contaminant profiles in Southeast Asian immigrants consuming fish from polluted waters in northeastern Wisconsin. *Environmental Research*, 110, 33-39.

23. Xing G.H., Liang Y., Chen L.X., Wu S.C., Wong M.H. (2011). Exposure to PCBs, through inhalation, dermal contact and dust ingestion at Taizhou, China - A major site for recycling transformers. *Chemosphere*, 83, 605-611.

24. Hindersinn R.R. (1990). Historical aspects of polymer fire retardance. *ACS Symposium Series*, 425, 87-96.

25. WHO (1997): Environmental Health Criteria 192. Flame retardants: A general introduction. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

26. Alaee M., Arias P., Sjödin A., Bergman Å. (2003). An overview of commercially used brominated flame retardants (BFRs) and their applications, changes in their use pattern in different countries/regions over time and possible modes of release. *Environment International*, 29, 683-689.

27. Darnerud P.O. (2008). Brominated flame retardants as possible endocrine disrupters. *International Journal of Andrology*, 31, 152-160.

28. Alexander J., Benford D., Boobis A.R., Ceccatelli S., Cravedi J.-P., Di Domenico A., Doerge D., Dogliotti E., Edler L., Farmer P., Filipic M., Fink-Gremmels J., Furst P., Guerin T., Knutsen H.K., Machala M., Mutti A., Schlatter J.R., van Leeuwen R., Bergman A., Johansson N., Knutsen H., Merletti F., Papke O., Schrenk D., van Leeuwen S., Zeilmaker M., Bordajandi L.R., Scaravelli E. (2011). Scientific opinion on polybrominated diphenyl ethers (PBDEs) in food. *EFSA Journal*,

29. Darnerud P.O. (2003). Toxic effects of brominated flame retardants in man and in wildlife. *Environment International*, 29, 841-853.

30. WHO (1995): Environmental Health Criteria 172. Tetrabromobisphenol A and derivatives. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

31. Joseph DiGangi, Arlene Blum, Åke Bergman, Cynthia A.de Wit, Donald Lucas, David Mortimer, Arnold Schecter, Martin Scheringer, Susan D.Shaw, Thomas F.Webster (2011). San Antonio Statement on Brominated and Chlorinated Flame Retardants. *Environmental Health Perspectives*, 119, A11.

32. Stockholm Convention on Persistent Organic Pollutants (POPs): Status of ratification. (3-17-2011) http://chm.pops.int/Countries/StatusofRatification/tabid/252/language/en-US/Default.aspx

33. Christensen F.M., Eisenreich S.J., Rasmussen K., Sintes J.R., Sokull-Kluettgen B., van de Plassche E.J. (2011). European Experience in Chemicals Management: Integrating Science into Policy. *Environmental Science and Technology*, 45, 80-89.

34. de Bruijn J., Hansen B., Munn S. (2003). Role of the precautionary principle in the EU risk assessment process on industrial chemicals. *Pure and Applied Chemistry*, 75, 2535-2541.

35. Christensen, F, de Bruijn, J, Hansen, B, Munn, S, Sokull-Klüttgen, B, Pedersen, F (2003). Assessment Tools under the New European Union Chemicals Policy 41 Greenleaf Publishing, 5-19.

36. Pouillot A., Polla B., Polla A. (2009). REACH: impact on the US cosmetics industry? *Journal of Cosmetic Dermatology*, 8, 3-7.

37. Baber Z. (2010). What Technology Wants by Kevin Kelly. *Nature (London, U.K.)*, 468, 372-373.

38. European Commission: REACH. (2007) http://ec.europa.eu/environment/chemicals/reach/pdf/2007_02_reach_in_brief.pdf

39. OSPAR Commission (2002). Provisional Instruction Manual for the Dynamic Selection and Prioritisation Mechnaism for Hazardous Substances (DYNAMEC) ISBN 094695-96-0. OSPAR Commission.

40. UNEP (2002). Ridding the world of POPs: A guide to the Stockholm convention on persistant organic pollutants United Nations Environmental Programme Chemicals, Switzerland.

41. UN-ECE (1998). Draft protocol to the convention on long-range air pollution of persistant organic pollutants EB.AIR/12998/2 United

Nations Economic and Social Council, Economic Commission for Europe.

42. Pavan M. and Worth A.P.: Review of QSAR models for ready biodegradation. (2011) http://ecb.jrc.ec.europa.eu/documents/QSAR/QSAR_Review_Biodegradation.pdf

43. US EPA: Summary of the Toxic Substances Control Act. (2011) http://www.epa.gov/lawsregs/laws/tsca.html

44. CEPA: Canadian Environmental Protection Act, 1999: Persistence and Bioaccumulation Regulations. (3-23-2000) http://canadagazette.gc.ca/archives/p2/2000/2000-03-29/html/sor-dors107-eng.html

45. Japanese Ministry of Economy T.a.I., Ministry of Health L.a.W., and Ministry of the Environment: Amendment of the Chemical Substances Control Law. (2011) http://www.meti.go.jp/policy/chemical_management/english/files/Amendment_of_CSCL_JPN.pdf

46. OECD: Guidelines for the Testing of Chemicals. (2011) http://www.oecd-ilibrary.org/content/package/chem_guide_pkg-en

47. OECD: Ready Biodegradability. (1992) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

48. OECD: Inherent Biodegradability: Modified MITI Test (II). (1981) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

49. OECD: Inherent Biodegradability in Soil. (1981) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

50. OECD: Inherent Biodegradability: Zahn-Wellens/EVPA Test. (1992) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

51. OECD: Revised introduction to the OECD Guidelines for testing of chemicals, section 3, part 1. (2006) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

52. OECD: Simulation Test - Aerobic Sewage Treatment - A: Activated Sludge Units, B: Biofilms. (2001) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

53. OECD: Aerobic Mineralisation in Surface Water - Simulation Biodegradation Test. (2004) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

54. OECD: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater. (2008) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

55. OECD: Aerobic and Anaerobic Transformation in Soil. (2002) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

56. OECD: Aerobic and Anaerobic Transformatin in Aquatic Sediment Systems. (2002) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

57. Stamatelatou K., Antonopoulou G., Lyberatos G. (2011). Production of biogas via anaerobic digestion. *Woodhead Publishing Series in Energy*, 15, 266-304.

58. OECD: Anaerobic Biodegradability of Organic Compounds in Digested Sludge. (2006) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

59. OECD: Hydrolysis as a Function of pH. (2004) http://www.oecd-ilibrary.org/environment/test-no-111-hydrolysis-as-a-function-of-ph_9789264069701-en

60. OECD: Phototransformation of Chemicals on Soil Surfaces. (2002) http://www.oecd.org/dataoecd/44/41/2741541.pdf

61. OECD: Phototransformation of Chemicals in Water - Direct Photolysis. (2008) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x

62. Howard P.H., Banerjee S. (1984). Interpreting results from biodegradability tests of chemicals in water and soil. *Environmental Toxicology and Chemistry*, 3, 551-562.

63. Howard P.H. (2000). Biodegradation. *Handbook of Chemical Property Estimation Methods*, 281-310.

64. Green N., Bergman Å. (2005). Chemical reactivity as a tool for estimating persistance. *Environmental Science and Technology*, 39, 480A-486A.

65. Neilson AH, Allard A-S (2008): Environmental Degradation and Transformation of Organic Chemicals. CRC Press, Taylor & Francis Group.

66. Hasan S., Cho J.G., Sublette K.L., Pak D., Maule A. (1992). Porphyrin-catalyzed degradation of chlorinated phenols and nitro-substituted toluenes. *Journal of Biotechnology*, 24, 195-201.

67. You G., Sayles G.D., Kupferle M.J., Kim I.S., Bishop P.L. (1996). Anaerobic DDT biotransformation: enhancement by application of surfactants and low oxidation reduction potential. *Chemosphere*, 32, 2269-2284.

68. Schwarzenbach RP, Gschwend PM, Imboden DM (2003): Environmental organic chemistry. John Wiley & Sons, Inc., Hoboken, New Jersey.

69. Gerecke A.C., Hartmann P.C., Heeb N.V., Kohler H.P., Giger W., Schmid P., Zennegg M., Kohler M. (2005). Anaerobic Degradation of Decabromodiphenyl Ether. *Environmental Science and Technology*, 39, 1078-1083.

70. Robrock K.R., Korytar P., varez-Cohen L. (2008). Pathways for the anaerobic microbial debromination of polybrominated diphenyl ethers. *Environmental Science and Technology*, 42, 2845-2852.

71. Janssen D.B., Oppentocht J.E., Poelarends G.J. (2001). Microbial dehalogenation. *Current Opinion in Biotechnology*, 12, 254-258.

72. Rayne S., Ikonomou M.G., Whale M.D. (2003). Anaerobic microbial and photochemical degradation of 4,4'-dibromodiphenyl ether. *Water Research*, 37, 551-560.

73. He J., Robrock K.R., Alvarez-Cohen L. (2006). Microbial Reductive Debromination of Polybrominated Diphenyl Ethers (PBDEs). *Environmental Science and Technology*, 40, 4429-4434.

74. Smidt H., de Vos W.M. (2004). Anaerobic microbial dehalogenation. *Annual Review of Microbiology*, 58, 43-73.

75. Cord-Ruwisch R., Seitz H.J., Conrad R. (1988). The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. *Archives of Microbiology*, 149, 350-357.

76. Fernandez I., Frenking G., Uggerud E. (2010). Rate-Determining Factors in Nucleophilic Aromatic Substitution Reactions. *Journal of Organic Chemistry*, 75, 2971-2980.

77. Eriksson J., Eriksson L., Marsh G., and Bergman Å. (2003). A one step synthesis of all nona-brominated diphenyl ethers. *Organohalogen Compounds*, 61, 191-194.

78. Keum Y., Li Q., X (2005). Reductive debromination of polybrominated diphenyl ethers by zerovalent iron. *Environmental Science and Technology*, 39, 2280-2286.

79. Li A., Tai C., Zhao Z., Wang Y., Zhang Q., Jiang G., Hu J. (2007). Debromination of Decabrominated Diphenyl Ether by Resin-Bound Iron Nanoparticles. *Environmental Science and Technology*, 41, 6841-6846.

80. Tokarz J.A., Ahn M.Y., Leng J., Filley T.R., Nies L. (2008). Reductive debromination of polybrominated diphenyl ethers in anaerobic sediment and a biomimetic system. *Environmental Science and Technology*, 42, 1157-1164.

81. Yen J.H., Liao W.C., Chen W.C., Wang Y.S. (2009). Interaction of polybrominated diphenyl ethers (PBDEs) with anaerobic mixed bacterial cultures isolated from river sediment. *Journal of Hazardous Materials*, 165, 518-524.

82. Fenner K., Scheringer M., Hungerbuehler K. (2000). Persistence of Parent Compounds and Transformation Products in a Level IV Multimedia Model. *Environmental Science and Technology*, 34, 3809-3817.

83. Kern S., Singer H., Hollender J., Schwarzenbach R.P., Fenner K. (2011). Assessing Exposure to Transformation Products of Soil-Applied Organic Contaminants in Surface Water: Comparison of Model Predictions and Field Data. *Environmental Science and Pollution Research*, 45, 2833-2841.

84. Ng C.A., Scheringer M., Fenner K., Hungerbuhler K. (2011). A Framework for Evaluating the Contribution of Transformation Products to Chemical Persistence in the Environment. *Environmental Science and Technology*, 45, 111-117.

85. van Zelm R., Huijbregts M.A.J., van de Meent D. (2010). Transformation Products in the Life Cycle Impact Assessment of Chemicals. *Environmental Science and Technology*, 44, 1004-1009.

86. Lee L.K., He J. (2010). Reductive debromination of polybrominated diphenyl ethers by anaerobic bacteria from soils and sediments. *Applied and Environmental Microbiology*, 76, 794-802.

87. Bonin P.M.L., Edwards P., Bejan D., Lo C.C., Bunce N.J., Konstantinov A.D. (2005). Catalytic and electrocatalytic hydrogenolysis of brominated diphenyl ethers. *Chemosphere*, 58, 961-967.

88. Konstantinov A., Bejan D., Bunce N.J., Chittim B., McCrindle R., Potter D., Tashiro C. (2008). Electrolytic debromination of PBDEs in DE-83 (TM) technical decabromodiphenyl ether. *Chemosphere*, 72, 1159-1162.

89. Zeng X., Simonich S.L.M., Robrock K.R., Korytar P., Alvarez-Cohen L., Barofsky D.F. (2010). Application of a congener-specific debromination model to study photodebromination, anaerobic microbial debromination, and FE0 reduction of polybrominated diphenyl ethers. *Environmental Toxicology and Chemistry*, 29, 770-778.

90. Room and Haas: Sodium borohydride digest. (2003) http://www.dow.com/assets/attachments/industry/pharma_medical/chemical_reagents/reducing_agents/sodium_borohydride_digest.pdf

91. Gauthier L.T., Hebert C.E., Weseloh D.V.C., Letcher R.J. (2008). Dramatic Changes in the Temporal Trends of Polybrominated Diphenyl Ethers (PBDEs) in Herring Gull Eggs From the Laurentian Great Lakes: 1982-2006. *Environmental Science and Technology*, 42, 1524-1530.

92. Vane C.H., Ma Y.J., Chen S.J., Mai B.X. (2010). Increasing polybrominated diphenyl ether (PBDE) contamination in sediment cores from the inner Clyde Estuary, UK. *Environmental Geochemistry and Health*, 32, 13-21.

93. La Guardia M.J., Hale R.C., Harvey E. (2007). Evidence of Debromination of Decabromodiphenyl Ether (BDE-209) in Biota from

a Wastewater Receiving Stream. *Environmental Science and Technology*, 41, 6663-6670.

94. Kohler M., Zennegg M., Bogdal C., Gerecke A.C., Schmid P., Heeb N.V., Sturm M., Vonmont H., Kohler H.P., Giger W. (2008). Temporal Trends, Congener Patterns, and Sources of Octa-, Nona-, and Decabromodiphenyl Ethers (PBDE) and Hexabromocyclododecanes (HBCD) in Swiss Lake Sediments. *Environmental Science and Technology*, 42, 6378-6384.

95. Davis E.F., Stapleton H.M. (2009). Photodegradation Pathways of Nonabrominated Diphenyl Ethers, 2-Ethylhexyltetrabromobenzoate and Di(2-ethylhexyl)tetrabromophthalate: Identifying Potential Markers of Photodegradation. *Environmental Science and Technology*, 43, 5739-5746.

96. de Boer J., Allchin C., Law R., Zegers B., Boon J.P. (2001). Method for the analysis of polybrominated diphenylethers in sediments and biota. *Trends in Analytical Chemistry*, 20, 591-599.

97. Covaci A., Voorspoels S., de Boer J. (2003). Determination of brominated flame retardants, with emphasis on polybrominated diphenyl ethers (PBDEs) in environmental and human samples - a review. *Environment International*, 29, 735-756.

98. Covaci A., Voorspoels S. (2005). Optimization of the determination of polybrominated diphenyl ethers in human serum using solid-phase extraction and gas chromatography-electron capture negative ionization mass spectrometry. *Journal of Chromatography B Analytical Technologies in the Biomedical and Life Sciences*, 827, 216-223.

99. Kierkegaard A., Sellstroem U., McLachlan M.S. (2009). Environmental analysis of higher brominated diphenyl ethers and decabromodiphenyl ethane. *Journal of Chromatography A*, 1216, 364-375.

100. Gomara B., Herrero L., Gonzalez M.J. (2007). Feasibility of electron impact and electron capture negative ionization mass spectrometry for the trace determination of tri- to deca-brominated diphenyl ethers in human samples. *Analytica Chimica Acta*, 597, 121-128.

101. Hites R.A. (2008). Electron Impact and Electron Capture Negative Ionization Mass Spectra of Polybrominated Diphenyl Ethers and Methoxylated Polybrominated Diphenyl Ethers. *Environmental Science and Technology*, 42, 2243-2252.

102. Vetter W. (2001). A GC/ECNI-MS method for the identification of lipophilic anthropogenic and natural brominated compounds in marine samples. *Analytical Chemistry*, 73, 4951-4957.

103. La Guardia M.J., Hale R.C., Harvey E. (2006). Detailed Polybrominated Diphenyl Ether (PBDE) Congener Composition of the Widely Used Penta-, Octa-, and Deca-PBDE Technical Flame-retardant Mixtures. *Environmental Science and Technology*, 40, 6247-6254.

104. Rahm S., Green N., Norrgran J., Bergman Å. (2005). Hydrolysis of environmental contaminants as an experimental tool for indication of their persistency. *Environmental Science and Technology*, 39, 3128-3133.

105. Söderström G., Sellström U., de Wit C.A., Tysklind M. (2004). Photolytic debromination of decabromodiphenyl ether (BDE 209). *Environmental Science and Technology*, 38, 127-132.

106. Eriksson J., Rahm S., Green N., Bergman Å., Jakobsson E. (2004). Photochemical transformations of tetrabromobisphenol A and related phenols in water. *Chemosphere*, 54, 117-126.

107. Moreira Bastos P.M., Eriksson J., Bergman A. (2009). Photochemical decomposition of dissolved hydroxylated polybrominated diphenyl ethers under various aqueous conditions. *Chemosphere*, 77, 791-797.

108. Butt C.M., Diamond M.L., Truong J., Ikonomou M.G., ter Schure A.F.H. (2004). Spatial Distribution of Polybrominated Diphenyl Ethers in Southern Ontario as Measured in Indoor and Outdoor Window Organic Films. *Environmental Science and Technology*, 38, 724-731.

109. Bastos P.M., Eriksson J., Virdarson J., Bergman A. (2008). Oxidative transformation of polybrominated diphenyl ether congeners (PBDEs) and of hydroxylated PBDEs (OH-PBDEs). *Environmental Science and Pollution Research*, 15, 606-613.

110. Bastos P.M., Eriksson J., Green N., Bergman A. (2008). A standardized method for assessment of oxidative transformations of brominated phenols in water. *Chemosphere*, 70, 1196-1202.

111. Eriksson J., Green N., Marsh G., Bergman Å. (2004). Photochemical decomposition of 15 polybrominated diphenyl ether congeners in methanol/water. *Environmental Science and Technology*, 38, 3119-3125.

112. Granelli L., Eriksson J., Athanasiadou M., Bergman Å. (2011). Reductive debromination of nonabrominated diphenyl ethers by sodium borohydride and identification of octabrominated diphenyl ether products. *Chemosphere*, 82, 839-846.

113. Qiu Y., Eriksson J., Granelli L., Bergman A. (2009). An improved method for assessing relative nucleophilic substitution reactivities of polychlorinated benzenes. *Chemosphere*, 75, 78-82.

114. Ballschmiter K., Zell M. (1980). Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Composition of technical aroclor-and clophen-PCb mixtures. *Fresenius' Zeitschrift für analytische Chemie*, 302, 20-31.

115. Ballschmiter K., Mennel A., Buyten J. (1993). Long chain alkyl-polysiloxanes as non-polar stationary phases in capillary gas chromatography. *Fresenius' Journal of Analytical Chemistry*, 346, 396-402.

116. WHO (1994): Environmental Health Criteria 162. Brominated diphenyl ethers. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

117. Andersson Ö., Blomkvist G. (1981). Polybrominated aromatic pollutants found in fish in Sweden. *Chemosphere*, 10, 1051-1060.

118. Norén K. and Meironyté D. (1998). Contaminants in swedish human milk. Decreasing levels of organochlorine and increasing levels of organobromine compounds. *Organohalogen Compounds*, 38, 1-4.

119. Hale R.C., Alaee M., Manchester-Neesvig J.B., Stapleton H.M., Ikonomou M.G. (2003). Polybrominated diphenyl ether flame retardants in the North American environment. *Environment International*, 29, 771-779.

120. Hites R., Foran J., Schwager S., Knuth B., Hamilton C., and Carpenter D. (2004). Global assessment of polybrominated diphenyl ethers in farmed and wild salmon. *Organohalogen Compounds*, 66, 3873-3876.

121. Norstrom R.J., Simon M., Moisey J., Wakeford B., Weseloh D.V.C. (2002). Geographic distribution (2000) and temporal trends (1981-2000) of brominated diphenyl ethers in Great Lakes herring gull eggs. *Environmental Science and Technology*, 36, 4783-4789.

122. She J., Petreas M., Winkler J., Visita P., McKinney M., Kopec D. (2002). PBDEs in the San Fransisco bay area: measurements in harbor seal blubber and human breast adipose tissue. *Chemosphere*, 46, 697-707.

123. Fängström B., Athanassiadis I., Odsjö T., Noren K., Bergman Å. (2008). Temporal trends of polybrominated diphenyl ethers and hexabromocyclododecane in milk from Stockholm mothers, 1980-2004. *Molecular Nutrition and Food Research*, 52, 187-193.

124. de Wit C. (2002). An overview of brominated flame retardants in the environment. *Chemosphere*, 46, 583-624.

125. Cox P., Efthymiou P. (2003). Directive 2003/11/EC of the European parliament and of the council of February 6 2003 amending for the 24th time Council Directive 76/669/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether). *Official Journal of the European Union*, OJ L 42, 45-46.

126. Great Lakes Flame Retardants: Regulatory Updates - United States. (2005) http://www.e1.greatlakes.com/fr/common/jsp/index.jsp

127. EU T.C.o.J.o.t.E.C.: Directive 2002/95/EC-Electrical and electronic equipment - Restriction of use of certain hazardous substances - Decabromodiphenyl ether ('DecaBDE') - Commission Decision 2005/717/EC - Exemption of DecaBDE from the prohibition on use - Actions for annulment - Commission's implementing powers - Infringement of the enabling provision. (2008) http://curia.europa.eu

128. BSEF: Brominated Flame Retardant DecaBDE Fact Sheet. (2010) http://www.bsef.com/uploads/doc/BSEF_Deca-BDE_08-10f.pdf

129. Wania F., Dugani C.B. (2003). Assessing the long-range transport potential of polybrominated diphenyl ethers: A comparison of four multimedia models. *Environmental Toxicology and Chemistry*, 22, 1252-1261.

130. Stapleton H.M., Brazil B., Holbrook R.D., Mitchelmore C.L., Benedict R., Konstantinov A., Potter D. (2006). In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile

rainbow trout and common carp. *Environmental Science and Technology*, 40, 4653-4658.

131. Law K., Halldorson T., Danell R., Stern G., Gewurtz S., Alaee M., Marvin C., Whittle M., Tomy G. (2006). Bioaccumulation and trophic transfer of some brominated flame retardants in a Lake Winnipeg (Canada) food web. *Environmental Toxicology and Chemistry*, 25, 2177-2186.

132. Muir D.C.G., Backus S., Derocher A.E., Dietz R., Evans T.J., Gabrielsen G.W., Nagy J., Norstrom R.J., Sonne C., Stirling I., Taylor M.K., Letcher R.J. (2006). Brominated Flame Retardants in Polar Bears (Ursus maritimus) from Alaska, the Canadian Arctic, East Greenland, and Svalbard. *Environmental Science and Technology*, 40, 449-455.

133. Streets S.S., Henderson S.A., Stoner A.D., Carlson D.L., Simcik M.F., Swackhamer D.L. (2006). Partitioning and Bioaccumulation of PBDEs and PCBs in Lake Michigan. *Environmental Science and Technology*, 40, 7263-7269.

134. Vonderheide A.P., Mueller K.E., Meija J., Welsh G.L. (2008). Polybrominated diphenyl ethers: Causes for concern and knowledge gaps regarding environmental distribution, fate and toxicity. *Science of the Total Environment*, 400, 425-436.

135. BSEF (2008) www.bsef.com

136. Mörck A., Hakk H., Örn U., Klasson Wehler E. (2003). Decabromodiphenyl ether in the rat: absorption, distribution, metabolism, and excretion. *Drug Metabolism and Disposition*, 31, 900-907.

137. Thuresson K., Bergman Å., Jakobsson K. (2005). Occupational exposure to commercial decabromodiphenyl ether in workers manufacturing or handling flame retarded rubber. *Environmental Science and Technology*, 39, 1980-1986.

138. Van den Steen E., Covaci A., Jaspers V.L.B., Dauwe T., Voorspoels S., Eens M., Pinxten R. (2007). Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (Sturnus vulgaris). *Environmental Pollution*, 148, 648-653.

139. Morita M. (1977). Chlorinated benzenes in the environment. *Ecotoxicology and Environmental Safety*, 1, 1-6.

140. WHO (1991). Environmental Health Criteria 128. Chlorobenzenes other than hexachlorobenzene.

141. WHO (1997): Environmental Health Criteria 195. Hexachlorobenzene. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

142. Hoh E., Zhu L., Hites R.A. (2005). Novel Flame Retardants, 1,2-Bis(2,4,6-tribromophenoxy)ethane and 2,3,4,5,6-Pentabromoethylbenzene, in United States' Environmental Samples. *Environmental Science and Technology*, 39, 2472-2477.

143. Hakk H., Larsen G., Bowers J. (2004). Metabolism, tissue disposition, and excretion of 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) in male Sprague-Dawley rats. *Chemosphere*, 54, 1367-1374.

144. Verreault J., Gebbink W.A., Gauthier L.T., Gabrielsen G.W., Letcher R.J. (2007). Brominated Flame Retardants in Glaucous Gulls from the Norwegian Arctic: More Than Just an Issue of Polybrominated Diphenyl Ethers. *Environmental Science and Technology*, 41, 4925-4931.

145. Wu J.P., Guan Y.T., Zhang Y., Luo X.J., Zhi H., Chen S.J., Mai B.X. (2011). Several current-use, non-PBDE brominated flame retardants are highly bioaccumulative: Evidence from field determined bioaccumulation factors. *Environment International*, 37, 210-215.

146. Stapleton H.M., Allen J.G., Kelly S.M., Konstantinov A., Klosterhaus S., Watkins D., McClean M.D., Webster T.F. (2008). Alternate and new brominated flame retardants detected in US house dust. *Environmental Science and Technology*, 42, 6910-6916.

147. Julander A., Karlsson M., Hagstroem K., Ohlson C.G., Engwall M., Bryngelsson I.L., Westberg H., van Bavel B. (2005). Polybrominated diphenyl ethers-plasma levels and thyroid status of workers at an electronic recycling facility. *International Archives of Occupational and Environmental Health*, 78, 584-592.

148. Sjödin A., Carlsson H., Thuresson K., Sjölin S., Bergman Å., Östman C. (2001). Flame retardants in indoor air at an electronics recycling plant and at other work environments. *Environmental Science and Technology*, 35, 448-454.

149. Karlsson M., Ericson I., van Bavel B., Jensen J.K., Dam M. (2006). Levels of brominated flame retardants in Northern Fulmar (Fulmarus glacialis) eggs from the Faroe Islands. *Science of the Total Environment*, 367, 840-846.

150. Nomeir A.N., Markham P.M., Ghanayem B.I., Chadwick M. (1993). Disposition of the flame retardant 1,2-bis(2,4,6-tribromophenoxy)ethane. *Drug Metabolism and Disposition*, 21, 209-214.

151. Koster P., Debets F.M.H., Strik J.J.T.W. (1980). Porphyrinogenic action of fire retardants. *Bulletin of Environmental Contamination and Toxicology*, 25, 313-315.

152. Harju, M, Heimstad, ES, Herzke, D, Sandanger, T, Posner, S, Wania, F (2008). Emerging "new" brominated flame retardats in flame retarded products and the environment.

153. Hassenkloever T., Predehl S., Pilli J., Ledwolorz J., Assmann M., Bickmeyer U. (2006). Bromophenols, both present in marine organisms and in industrial flame retardants, disturb cellular Ca2+ signaling in neuroendocrine cells (PC12). *Aquatic Toxicology*, 76, 37-45.

154. Balabanovich A.I., Luda M.P., Operti L. (2004). GC/MS identification of pyrolysis products from 1,2-Bis(2,4,6-tribromophenoxy)ethane. *Journal of Fire Sciences*, 22, 269-292.

155. European Commission (2001). White paper. Strategy for a future Chemials Policy COM(2001) 88 final, 2001-02-27, Brussels, Belgium.

156. Lam J.C.W., Lau R.K.F., Murphy M.B., Lam P.K.S. (2009). Temporal Trends of Hexabromocyclododecanes (HBCDs) and Polybrominated Diphenyl Ethers (PBDEs) and Detection of Two Novel Flame Retardants in Marine Mammals from Hong Kong, South China. *Environmental Science and Technology*, 43, 6944-6949.

157. Arias P. (2001). Brominated flame retardants - An overview. *The Second International Workshop on Brominated Flame Retardants, BFR 2001*17-20.

158. Huwe J.K., Smith D.J. (2007). Accumulation, Whole-Body Depletion, and Debromination of Decabromodiphenyl Ether in Male Sprague-Dawley Rats Following Dietary Exposure. *Environmental Science and Technology*, 41, 2371-2377.

159. Thomas G., Moss S.E.W., Asplund L., Hall A.J. (2005). Absorption of decabromodiphenyl ether and other organohalogen chemicals by grey seals (*Halichoerus grypus*). *Environmental Pollution*, 133, 581-586.

160. Luo X.J., Zhang X.l., Liu J., Wu J.P., Luo Y., Chen S.J., Mai B.X., Yang Z.y. (2009). Persistent Halogenated Compounds in Waterbirds from an e-Waste Recycling Region in South China. *Environmental Science and Technology*, 43, 306-311.

161. Kierkegaard A., Björklund J., Fridén U. (2004). Identification of the Flame Retardant Decabromodiphenyl Ethane in the Environment. *Environmental Science and Technology*, 38, 3247-3253.

162. La Guardia M.J., Hale R.C., Harvey E., Chen D. (2010). Flame-Retardants and Other Organohalogens Detected in Sewage Sludge by Electron Capture Negative Ion Mass Spectrometry. *Environmental Science and Technology*, 44, 4658-4664.

163. Hardy M.L. (2004). A comparison of the fish bioconcentration factors for brominated flame retardants with their nonbrominated analogues. *Environmental Toxicology & Chemistry*, 23, 656-661.

164. Hardy M.L., Margitich D., Ackerman L., Smith R.L. (2002). The subchronic oral toxicity of ethane, 1,2-bis(pentabromophenyl) (Saytex 8010) in rats. *International Journal of Toxicology*, 21, 165-170.

165. Nakari T., Huhtala S. (2010). In vivo and in vitro toxicity of decabromodiphenyl ethane, a flame retardant. *Environmental Toxicology*, 25, 333-338.