



CONTROL OF HAZARDOUS SUBSTANCES
IN THE BALTIC SEA REGION

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WP3 INNOVATIVE APPROACHES TO CHEMICALS CONTROL OF HAZARDOUS SUBSTANCES

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Abbreviations

BOD	Biological oxygen demand
BPA	bisphenol A
BSAP	Baltic Sea Action Plan
Cd	Cadmium
COD	Chemical oxygen demand
CVAFS	Cold vapour atomic fluorescence spectroscopy
DBT	Dibutyltin cation
decaBDE	Decabromodiphenyl ether
DOT	Dioctyltin cation
EQS	Environmental quality standard
EROD	Ethoxyresorufin-O-deethylase
GC/ECNI-MS	Gas chromatographic electron capture negative ionization mass spectrometry
GC-ECD	Gas chromatography combined with electron capture detector
GC-HRMS	Gas chromatography combined with high resolution mass spectrometry
GC-MS/MS	Gas chromatography combined with tandem mass spectrometry
GC-MS-NCI	Gas chromatography combined with mass spectrometry–negative ion chemical ionization detection
HBCD	Hexabromocyclododecane
Hg	Mercury
HLB	Hydrophilic lipophilic balanced
LC-MS/MS	Liquid chromatography combined with tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MBT	Monobutyltin cation
MCCP	Medium-chain chlorinated paraffin
MOT	Monooctyltin cation
MTBE	Methyl tertbutyl ether
MWWTP	Municipal wastewater treatment plant
NH ₄ -N	Ammonium nitrogen
NP	Nonylphenols
NPE	Nonylphenol ethoxylates
OCDD	Octachlorodibenzo-p-dioxin
OCDF	Octachlorodibenzofuran
octaBDE	Octabromodiphenyl ether
OP	Octylphenol
OPE	Octylphenol ethoxylate
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxin
PCDF	Polychlorinated dibenzofuran
pentaBDE	Pentabromodiphenyl ether
PFDA	Perfluoro-n-decanoic acid
PFH _x A	Perfluoro-n-hexanoic acid
PFOA	Perfluorooctanoic acid

PFOS	perfluorooctane sulphonic acid
PO4-P	Phosphate phosphorus
SCCP	Short-chain chlorinated paraffin
SPE	Solid phase extraction
SS	Suspended solids
TBT	Tributyltin
TCyT	Tricyclohexyltin cation
TEF	Toxic equivalency factor
TEQ	TCDD toxic equivalent
TOC	Total organic carbon
Tot N	Total nitrogen
Tot P	Total phosphorous
TPhT	Triphenyltin compound
TTBT	Tetrabutyltin
UFLC	Ultra fast liquid chromatograph
WFD	Water Framework Directive
WHO TEQ	TEQ calculated with WHO-TEF
WWTP	Wastewater treatment plant

Laboratories

Bioplan GmbH	Germany
EERC	Estonian Environmental Research Centre
Eurofins	Denmark
Eurofins GfA	GmbH in Germany
EPA	Environmental Protection Agency, Lithuania
IETU	Institute for Ecology of Industrial Areas, Poland
IVL	Swedish Environmental Research Institute
LHEI	Latvian Institute of Aquatic Ecology
IMN	Institute of Non-Ferrous Metals in Gliwice, Poland
SYKE	Finnish Environment Institute
THL	National Institute for Health and Welfare, Finland

1 Introduction

Control of Hazardous Substances in the Baltic Sea or COHIBA –project includes all Baltic Sea countries except Russia, the objective of COHIBA is to support the implementation of the HELCOM Baltic Sea Action Plan (BSAP) with regard to hazardous substances by developing joint actions to reach the goal of “Baltic Sea with life undisturbed by hazardous substances”. The 11 substances/substance groups identified in the BSAP as being of special concern to the Baltic Sea are also the focus of this project. The COHIBA project will last three years (2009-2012).

The COHIBA project aims to identify the most important sources of 11 hazardous substances of special concern. The project analyses flow patterns of these substances and quantifies their releases and inputs to the Baltic Sea. The project will also develop innovative and cost-effective evaluation practices of effluent ecotoxicity, based on the Whole Effluent Assessment (WEA) approach. The target is also to define toxicity-based discharge limits, a threshold toxicity, to effluents discharged into receiving waters in the Baltic Sea region. In addition, the aim is to share knowledge about best practices within the participating countries and to assist authorities and industries to control hazardous substances. The project will enhance the capability of the Baltic Sea countries to implement their international obligations under the forthcoming European Marine Strategy and the Water Framework Directive. It also contributes to national implementation programmes to reach the cessation targets for HELCOM/EU priority hazardous substances by 2020.

The COHIBA project consists of following work packages (WPs):

WP1	Project management and administration (lead by Finnish Environment Institute SYKE)
WP2	Communication and Information (lead by HELCOM Secretariat)
WP3	Innovative approaches to chemical controls of hazardous substances (lead by SYKE)
WP4	Identification of sources and estimation of inputs/impacts on the Baltic Sea (lead by Swedish Environmental Research Institute IVL)
WP5	Cost effective management options to reduce discharges, emissions and losses of hazardous substances (lead by Federal Environment Agency of Germany UBA)
WP6	Capacity building and knowledge transfer (lead by Baltic Environmental Forum Estonia BEF-EE)

The target of WP3

The target of WP3 was to contribute to the identification of sources for the 11 hazardous substances in BSAP by performing screening in municipal and industrial waste waters, landfill leachates and storm waters, in all participating countries. WP3 also aimed for developing joint evaluation of ecotoxicity of the effluents and for recommending PBT (persistent, bioaccumulating, toxic)-based discharge limit values based on the WEA approach for the Baltic Sea region. Currently most of the restrictions concerning discharges and emissions are based on the determination of chemical concentrations. However, the majority of effluents comprise a mixture of chemicals. It is impossible to identify all these substances and their transformation products or to determine the effects of all individual substances or their synergistic interactions in the environment. Thus there is a need to regulate discharges on the basis of direct assessments of their biological effects, to complement the chemical analyses. Also, an important aim of WP3 was to harmonise the chemical and ecotoxicological methods in the Baltic Sea region serving also EU WFD and REACH requirements. The results will be used as input to the integrated HELCOM assessment of hazardous substances and of the sources for the substances of concern to the Baltic Sea. ¹

¹ Detailed results and discussions are in the national reports of participating countries.

Detailed descriptions of analytical methods can be found in the national reports and ring-test reports (www.cohiba-project.net). In these reports the performing laboratories are also indicated.

2 Monitoring program

2.1 Sampling locations and sampling program

The participating countries were supposed to chose sampling sites of municipal and industrial wastewater effluents, landfill leachates and storm waters discharged into the Baltic Sea. The aim was to cover different types of loads to the Baltic Sea from all the participating countries and to take samples at the same point in time.

According to the project plan, waste water effluents were sampled six times during the project. Storm water, landfill leachate and municipal sewage sludge were sampled twice; once during the cold and once during the warm season. Effluent samples were analysed both for the selected chemicals as well as for toxicity, while the municipal sewage sludge was analysed for chemicals only.

However, the sampling program differed between the countries. Detailed information on the sampling locations and sampling program in each country can be found in the national reports (www.cohiba-project.net).

The first two sampling rounds of effluents concentrated only on toxicity, the next four rounds both on toxicity and chemical analyses, and the last two sampling rounds were for chemical analyses only. The idea in starting with toxicity tests was to leave the option to change the sampling points, if there where e.g. no toxicity during the first two sampling rounds. However, it was decided to keep original sampling points throughout the whole project. Only Estonia and Germany changed one of their sampling points.

Information and some basic data from the treatment plants are shown in tables 2.1. and 2.2. Effluent treatment plants in these tables are grouped by the treatment process.

Table 2.1. Description of the wastewater treatment plants used as case studies in COHIBA WP3 grouped by type of treatment (MWWTP, municipal wastewater treatment plant).

CODE	Sampling	Year of establ.	Type of treatment	Flow-rate (m ³ /d)	Approx. Retention time	Approx. Population equivalents	Domestic: Industrial
DK_MWWTP1	24 h adjusted to the flowrate, two days combined	1980 (renovated in 1997)	Mechanical, Biological, Chemical, Improved Nitrogen and Phosphorus removal	180 000	24 h	750 000	90:10
DK_MWWTP2	24 h adjusted to the flowrate, two days combined	1930 (renovated continuously, latest in 1996)	Mechanical, Biological, Chemical, Improved Nitrogen and Phosphorus removal	75 000	24 h	350 000	85:15
EE_MWWTP1	24 h adjusted to the time	2009	Mechanical, Biological, Chemical, Improved Nitrogen and Phosphorus removal	16000	8-12 h	126 936	
EE_MWWTP2	24 h adjusted to the flowrate	2005	Mechanical, Biological, Chemical, Improved Nitrogen and Phosphorus removal	7240		55 559	
FI_MWWTP1	24 h adjusted to the flowrate	2008	Mechanical, Chemical, Biological, Improved Nitrogen removal	120 000		280 000	93:7
FI_MWWTP2	24 h adjusted to the flowrate	1969	Mechanical, Chemical, Biological, Improved Nitrogen removal	106 314	14 h	295 000	92:8
FI_MWWTP3	24 h adjusted to the flowrate	1994	Mechanical, Chemical, Biological, Improved Nitrogen removal	270 000	24 h	780 000	85:15
SE_MWWTP1	24 h adjusted to the flowrate	1941	Mechanical, Chemical, Biological, Improved Nitrogen removal	239 000		656 000	
SE_MWWTP3	24 h adjusted to the flowrate		Mechanical, Chemical, Biological, Improved Nitrogen removal	102 800		340 000	82:18
SE_MWWTP4	24 h adjusted to the flowrate		Mechanical, Chemical, Biological, Improved Nitrogen removal	19 000		67 600	73:27
DE_MWWTP1	24 h adjusted to time	1995	Mechanical, Chemical, Biological, Improved Nitrogen removal	39 717	41 h	398 408	72: 28
DE_MWWTP2	24 h adjusted to time	1997 (improved phosphorus removal); 2001 (improved nitrogen removal)	Mechanical, Chemical, Biological, Improved Nitrogen removal	9 718	3,5 h	100 000	53:47
SE_MWWTP2	24 h adjusted to the flowrate		Mechanical, Chemical, Biological	43000		131 800	85:15
PL_MWWTP2	24 h adjusted to flowrate	1976, 1999	Mechanical, Biological, Improved Nitrogen removal	96 105	48 h	573 720	93:7
PL_MWWTP3	24 h adjusted to flowrate	1994, 2006	Mechanical, Biological	53 569	48 h	420 000	95:5
PL_MWWTP1	24 h adjusted to flowrate	1997	Mechanical, Biological	10 812	48 h	99 100	95:5
LT_MWWTP1	Grab sample	1978	Mechanical, Biological	2735	35 h	21452	96:4
LT_MWWTP2	Grab sample	1988	Mechanical, Biological	3342		20945	90:10
LV_MWWTP1	24 h adjusted to time	1991	Mechanical, Biological	165 000	24h	717 371	95:5
LV_MWWTP2	24 h adjusted to time	1972/1998 (reconstruction)	Mechanical, Biological	55 000	24 h	90 000	95:5

Table 2.2. Description of the wastewater treatment plants used as case studies in COHIBA WP3 grouped by country and by type of treatment (IWWTP, industrial wastewater treatment plant).

CODE	Sampling	Year of establ.	Type of treatment	Flow-rate (m ³ /d)	Approx. Retention time	Approx. Population equivalents	Domestic: Industrial	Type of industry (and wastewater)
FI_IWWTP1	24 h time adjusted composite sample		Mechanical, Chemical, Biological	900	168 h		0:100	Oil refinery
EE_IWWTP3	24 h adjusted to the flowrate	2000	Mechanical, Biological with Nitrogen removal	374	13 h	7 873		Leather industry
EE_IWWTP4a	24 h adjusted to time	1981	Mechanical, Biological	866		5 574		Shipyard
EE_IWWTP4b	24 h adjusted to time	1980	Mechanical, Biological	1600		12 305		Shipyard
LT_IWWTP1	Grab sample	2003	Mechanical, Biological	373	374 h		82:18	PET plant
LT_IWWTP2	Grab sample	1959	Mechanical, Biological	1016			10:90	Oil refinery
LV_IWWTP1	Grab sample	1968	Mechanical, Biological	8 652			0:100	Metallurgical industry
LV_IWWTP2	Grab sample	1978	Mechanical, Biological	5000		1 000	0:100	Pharmaceutical industry
PL_IWWTP1	24 h adjusted to flowrate	1971	Mechanical, Chemical	498,6	4-6 h		0:100	Coal power plant
DE_IWWTP2	2 h Grab sample	2001	Groundwater tapping, filtration (gravel), softening, filtration via reverse osmosis	274	4-6 h		0:100	Woodworking industry
DE_IWWTP1	2 h Grab sample	1993	Flocculation, Sedimentation	642	30 h		0:100	Fishery sector / Coal power plant
DK_IWWTP1	Flowproportional combined	-	Sedimentation, pH-neutralisation	85	24-48 h	-	0:100	Waste Incineration Plant (water used to cool the slag)
DK_IWWTP2	Grab samples	-	No treatment	820	0 h	-	0:100	Power Plant (Concentrate from desalination of second rate water)

Storm water, landfill leachate and municipal sewage sludge were sampled twice; once during the cold and once during the warm season. Only exception was Estonia, where the both storm water samples we collected in spring, one before the snow cover had begun to melt and the other right after it. Water samples were analysed both for the selected target substances as well as for toxicity and municipal sludge only for chemicals (Table 2.3. and 2.4.).

Table 2.3. Description of the landfill sites used as case studies in COHIBA WP3 grouped by type of treatment.

Sampling	Landfill	Year of establishment	Type of treatment	Flow-rate (m ³ /d)	Approx. Retention time	Receiving water
Grab sample	SE		Biological, Improved Nitrogen removal, phyto-remediation system	56		Middle Sweden
24 h composite adjusted to time	EE	2000	Biological, Biological Nitrogen removal			River, 50 km from the shoreline, Gulf of Riga
Grab sample	LV	2004	Biological, SBR	280		River Daugava
Grab sample	DK	1878-1969	Biological, sandfilter, oxygen	55		MWWTP
Grab sample	DE	1996-2011	Biological, Chemical (ozonize)	35		surface water
24 h composite adjusted to time	FI	1987	None	1224	-	MWWTP
Grab sample	LT	1986-2007 (closed in 2008)	none	-	-	Drainage channel (approx. 9 km from the Curonian lagoon)
Composite from 5 different areas	PL	1977 -2007	None	18		MWWTP
Grab sample	DK	1960-1970	None	30		MWWTP

SBR = Sequence Batch Reactor

Table 2.4. Description of the storm water sites used as case studies in COHIBA WP3 grouped by type of treatment.

Sampling	Stormwater	Type of treatment	Drainage area (ha)	Flow-rate (m ³ /h)	Receiving water
Flow proportional	DK	Filtration, UV		44	North end of Copenhagen Harbour
Flow proportional	DK	Sand trap, oil trap, filtration		230	Trench in the district Ørestaden
Grab sample	DE	Sand trap	5		Wismar harbour
Grab sample	DK	None		50	South end of Copenhagen Harbour
Precipitation proportional	DK	None	2.9	2.2	Copenhagen Harbour
Grab sample	FI	None	130	0,43-4,9	Porolahti creek
Grab sample	SE	None			Stockholm area Lake Mälaren Årstaviken
Grab sample	EE	None			20 m from the shoreline, Gulf of Finland
Grab sample	LV	None	195.7	0.7	River Daugava, 6 km from the shoreline, Gulf of Riga
Grab sample	LT	None	527	0,25 -0,72	Smiltele stream (approx. 2,5 km from the Curonian lagoon)
Grab sample	PL	None	5.8	89.91	Harbour (Szczecin) basins in north-western part of Poland

2.2 Sampling

Wastewater effluent and landfill leachate samples were planned to take as 24 h composite samples adjusted to the flow rate or time. However, this procedure was not possible in all cases. Detailed information on the sampling procedures in each country can be found in the national reports (www.cohiba-project.net). There were special requirements for some analytes and analysing laboratories gave the relevant instruction in those cases. Storm water and sludge samples were taken as agreed within WP3. Sampling of storm water was started an hour after the start of the rain.

Right after the sampling, within the same day, samples were brought into the laboratory, mixed properly and dealt for different tests and analyses. For chemical analyses samples were bottled ac-

according to the instructions of the analysing laboratories. The samples for foreign laboratories were packed, according to the instructions of the laboratories and transported by air cargo.

For biotests, samples were divided into subsamples in plastic bottles. It was agreed to freeze the samples immediately after bottling to ensure that all samples would be handled similarly and results would be comparable. However, all countries did not follow these instructions.

3 Basic effluent parameters

Analyses of the basic effluent parameters (Table 3.1.) were partly conducted by the COHIBA partner laboratories and partly the results were received from cooperation partners.

Table 3.1. Basic parameters monitored in case studies.

flow rate	pH
biological oxygen demand (BOD)	total phosphorus
chemical oxygen demand (COD _{Cr})	total nitrogen
suspended solids	conductivity

Detailed information of the basic data of countries' treatment plants and the results of the basic effluent parameters are available in partners' National reports (www.cohiba-project.net).

The flow rate, in different treatment plants varied, due to the size, handling capacity and seasonal fluctuations tables 2.1. and 2.2.

Based on the available permitting values, on the COHIBA samplings, there were occasional exceedings in the permitted values of the basic parameters (see national reports) and the annual monitoring data showed significant variations. This is an indication that treatment capacity was exceeded occasionally. Minimum and maximum value of the basic parameters of all municipal and all industrial effluents studies are expressed in table 3.2. and those of landfill and storm water samples in table 3.3.

Table 3.1. The range of basic parameter results of all effluent samples, of municipal (MWWTP) and industrial (IWWTP) treatment plants.

Plant	BOD (mg/l)	COD _{Cr} (mg/l)	Suspended solids (mg/l)	pH	Tot.P (mg/l)	Tot.N (mg/l)	Efficiency (BOD ₇ , %)	Efficiency (N _{tot} , %)	Efficiency (P _{tot} , %)	Conduct. mS/m
MWWTP	2 - 19	24 - 113	3 - 12	6.7 - 7.7	0.1 - 2.9	4 - 41	95 - 99	10 - 90	79 - 98	60 - 2008
IWWTP	1 - 67	14 - 249	1 - 13	6.8 - 9.0	0.1 - 3.3	1 - 38	96 - 99	79 - 84	83 - 99	671 - 2470

Table 3.2. The range of basic parameter results of all landfill and storm water samples.

	TOC (mg/l)	BOD (mg/l)	COD _{cr} (mg/l)	Suspended solids (mg/l)	pH	Tot.P (mg/l)	Tot.N (mg/l)	conductivity
Landfill	< 1.01 - 955	5.6 - 610	18 - 2088	< 1.8 - 7936	4.7-8.8	0.02 - 25	6.5 - 2236	3.2 - 6650
Storm water	5.5 - 15		24 - 465	4.8 - 221	7.2 - 7.9	0.04 - 80	1.1 - 1500	15 - 2510

4 Chemical analyses

4.1 Metals

4.1.1 Cadmium, Cd

Cadmium is a naturally occurring minor element, one of the metallic components in the earth's crust and oceans, and present everywhere in our environment. Cadmium is recognized to produce toxic and carcinogenic effects on humans. Long-term occupational exposure can cause adverse health effects on the lungs and kidneys.

In COHIBA study, all countries analysed their own samples for cadmium, only Swedish and German samples were analysed by Polish laboratory IETU Institute for Ecology of Industrial Areas.

Analytical method for effluents, storm water and landfill leachate

The analytical methods for effluents, storm water and landfill leachate varied between laboratories. Three laboratories (Estonia, Denmark, and Finland) used inductively coupled plasma mass spectrometric method (ICP-MS) ISO 17294-2 and three laboratories (Poland, Lithuanian and Latvia) used atomic absorption spectrometric method with graphite furnace (GFAAS) ISO 15586.

Estonian laboratory changed the method during the project. Until the end of March 2010 there were used two different methods: inductively plasma optical emission spectrometric method (ICP-OES) ISO 11885 and atomic absorption spectrometric method with graphite furnace (GFAAS) ISO 15586. The Finnish laboratory used ICP-MS ISO 17294-2, but changed the LOQ in April 2010 (from 0.1µg/l to 0.05µg/l, Table 4.1.).

Table 4.1. LOQs (µg/l) for cadmium in municipal and industrial effluents, storm waters and landfill leachates.

Germany	Denmark	Estonia	Finland	Lithuania	Latvia	Poland	Sweden
0.2	0.05	0.1 – 0.02	0.1 – 0.05	0.05	0.3	0.2	0.2

Municipal waste water treatment plant

In total 114 municipal effluents were collected for analysis of cadmium. Cadmium was found in 7, i.e. 6% of municipal effluent samples (Table 4.2.)

Table 4.2. Number of samples and observed frequencies of cadmium concentrations above LOQ in municipal effluents.

	Number of samples	Frequency
Germany	12	0
Denmark	6	0
Estonia	12	5
Finland	18	1
Lithuania	12	0
Latvia	12	0
Poland	18	1
Sweden	24	0
TOTAL	114	7
%		6

In municipal effluents, cadmium was found in concentrations over the LOQ from three out of eight countries where it was measured – Estonia, Finland, and Poland. The maximum concentration (0,75 µg/l) was observed in Polish effluent (Figure 4.1.). However, it should be noted that the LOQ for Swedish samples were higher than, for example, Estonian and Finnish samples.

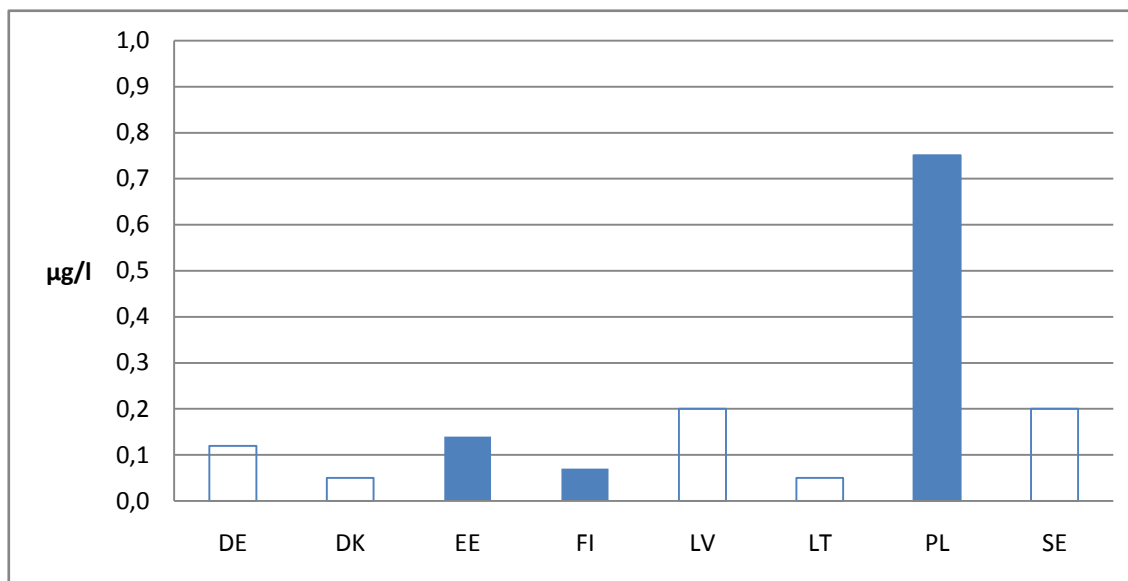


Figure 4.1. Maximum concentrations (µg/l) of cadmium found in municipal effluents. In cases where the maximum concentration was lower than LOQ, the bar is left blank.

Industrial waste water treatment plant

In total 57 industrial effluents were collected for analysis of Cadmium. Cadmium was found in 15, i.e. 26% of industrial effluent samples (Table 4.3.).

Table 4.3. Number of samples and observed frequencies of cadmium concentrations above LOQ in industrial effluents.

	Number of samples	Frequency
Germany	11	5
Denmark	4	2*
Estonia	12	6
Finland	6	0
Lithuania	6	0
Latvia	12	1
Poland	6	1
Sweden	0	0
TOTAL	57	15
%		26

* Danish results are shown over LOD

In industrial effluents, cadmium was found from five out of seven countries where it was measured – Germany, Denmark, Estonia, Latvia, and Poland. The maximum concentration of 4,0µg/l was found from German effluent (Figure 4.3.). There were no industrial effluent samples taken from Sweden, where all of the wastewater treatment plants were considered to be municipal.

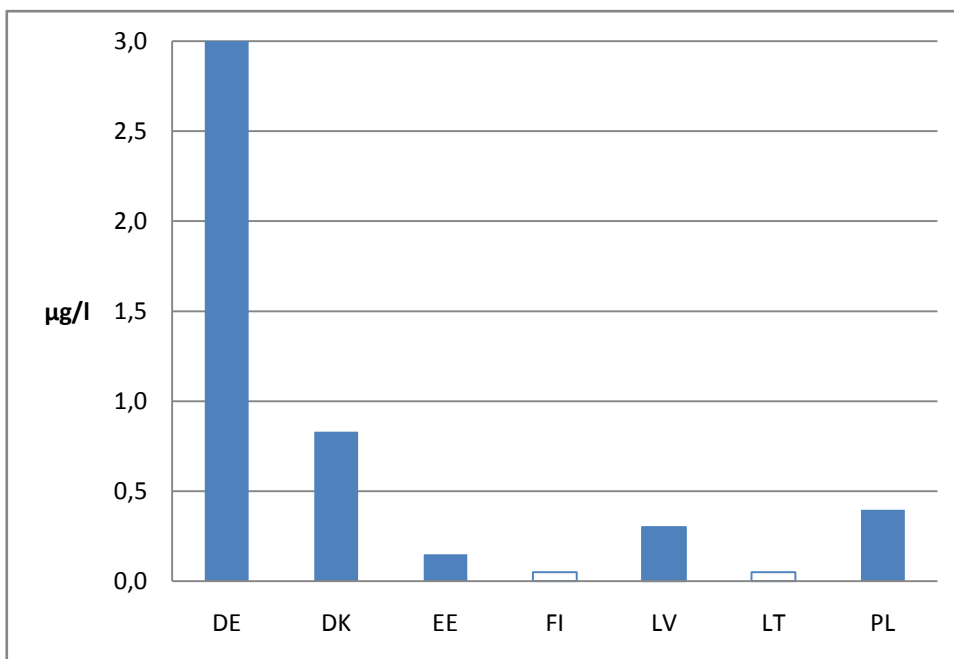


Figure 4.3. Maximum concentrations (µg/l) of cadmium found in industrial effluents. In cases where the maximum concentration was lower than LOQ, the bar is left blank.

Storm water

In total 15 storm water were collected for analysis of cadmium. Cadmium was found in 10, i.e. 67% of storm waters (Table 4.4).

Table 4.4. Number of samples and observed frequencies of cadmium concentrations above LOQ in storm waters.

	Number of samples	Frequency
Germany	2	2
Denmark	2	1*
Estonia	2	2
Finland	2	1
Lithuania	2	0
Latvia	1	1
Poland	2	2
Sweden	2	1
TOTAL	15	10
%		67

* Danish results are shown over LOD

In storm water samples, cadmium was found from all of the countries, except for Lithuania where both of the storm water samples were measured below the LOQ. The maximum concentration of 18.1µg/l was noted in Polish storm water (Figure 4.3.).

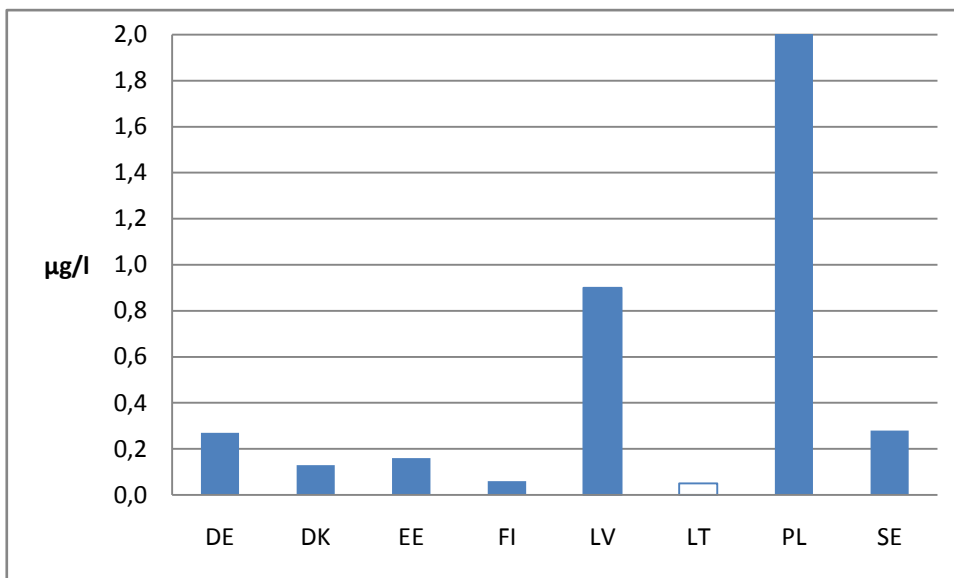


Figure 4.3. Maximum concentrations (µg/l) of cadmium found in storm waters. In case where the maximum concentration was lower than LOQ, the bar is left blank.

Landfill leachate

In total 14 landfill leachate samples were collected for analysis of cadmium. Cadmium was found in 6, i.e. 38% of landfill leachate samples (Table 4.5.).

Table 4.5. Number of samples and observed frequencies of cadmium concentrations above LOQ in landfill leachates.

	Number of samples	Frequency
Germany	2	0
Denmark	2	0
Estonia	2	0
Finland	2	2
Lithuania	2	0
Latvia	2	1
Poland	2	2
Sweden	2	1
TOTAL	16	6
%		38

In landfill leachates, cadmium was found in concentrations over the LOQ from three out of eight countries – Finland, Poland, and Swedish samples. The maximum concentration of 1.5 µg/l was observed in Polish landfill leachate (Figure 4.4.).

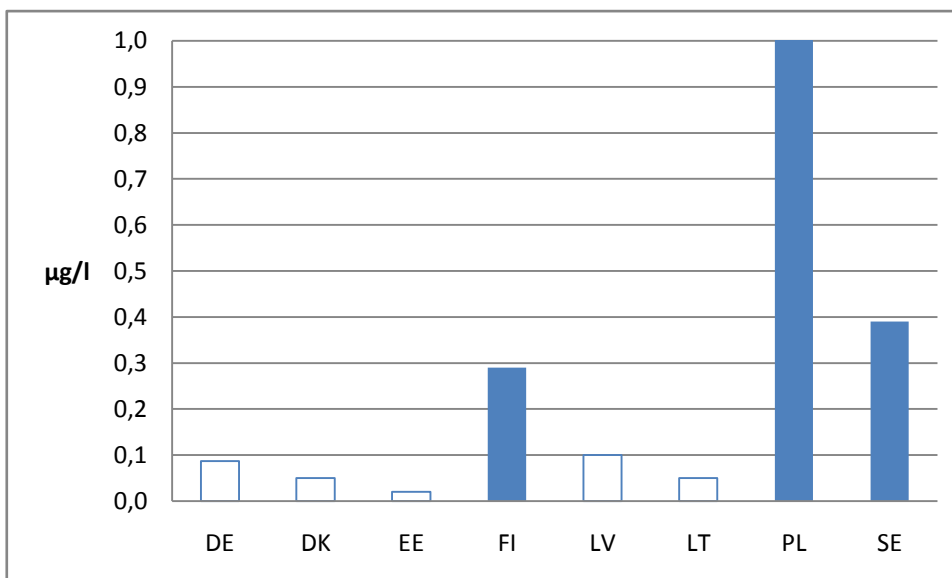


Figure 4.4. Maximum concentrations (µg/l) of cadmium found in leachates. In cases where the maximum concentration was lower than LOQ, the bar is left blank.

Analytical methods for sludge

The analytical methods for sludge samples varied between laboratories. Two laboratories (Denmark, Finland) used inductively coupled plasma mass spectrometric method (ICP-MS) ISO 17294-2, three laboratories (Poland, Lithuanian and Latvia) used atomic absorption spectrometric method with graphite furnace (GFAAS) ISO 15586 and one laboratory (Estonia) used inductively plasma optical emission spectrometric method (ICP-OES) ISO 11885. Limit of quantification (LOQ) varied between laboratories. In some cases LOQ were not informed (Table 4.6.).

In Denmark a small amount of sample is weighed into a plastic tube and added nitric acid and water for determination of Cd. The tube was sealed and placed in an autoclave where heating to 221 C°, ensures that the metals in the sample was dissolved. Finally the content of metals was measured with ICP-OES (Inductive coupled plasma – optic emission spectrometry).

Table 4.6. LOQs (mg/kg) for cadmium in sludge. The LOQ for Danish laboratories was not informed (n.i.). There were no sludge samples for Latvia.

Germany	Denmark	Estonia	Finland	Lithuania	Latvia	Poland	Sweden
0.2	n.i.	1.0	0.01	0.005	-	0.2	0.2

Sludge samples

Overall 18 sludge samples were collected for analysis of cadmium. Cadmium was found in 15, i.e. 83% of sludge samples (Table 4.7.).

Table 4.7. Number of samples and observed frequencies of cadmium concentrations above LOQ in sludge.

	Number of samples	Frequency
Germany	3	3
Denmark	4	4*
Estonia	3	0
Finland	2	2
Lithuania	2	2
Latvia	0	0
Poland	2	2
Sweden	2	2
TOTAL	18	15
%		83

* Danish results are shown over LOD

In sludge, cadmium was found from all the countries where it was measured, except from Estonia where Cd in all sludge samples were below the LOQ. However, it should be noted that the LOQ for Estonian sludge samples was higher than the results for several other countries where cadmium was found from, i.e. Germany, Finland, Lithuania and Sweden. The maximum concentration of 3.44mg/kg was noted in Polish sludge (Figure 2.5.).

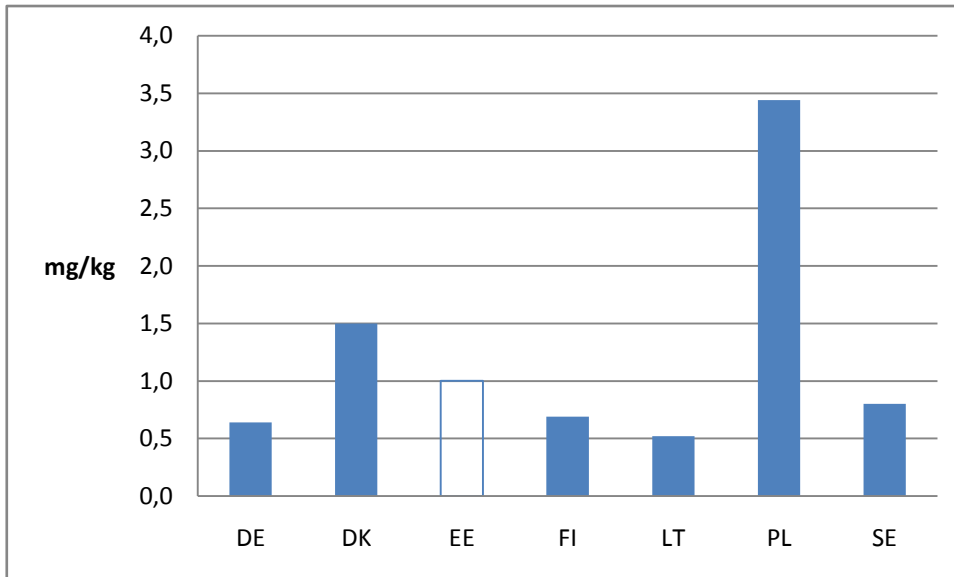


Figure 2.5. Maximum concentrations (mg/kg) of cadmium found in sludge samples. In case where the maximum concentration lower than the LOQ, the bar is left blank. Please note that there were no sludge samples taken from Latvia.

4.1.2 Mercury Hg

Mercury is toxic metallic element which causes harmful effects for environment and human health. The concentration of mercury in fish tissues can be 100 000 times higher than in ambient water. High levels of mercury concentration appear in fish and shellfish tissues due to bioaccumulation in the aquatic food chain. Mercury is released to the environment through natural and anthropogenic processes and is distributed globally. Anthropogenic activity during last century has released large amount of mercury in the environment. The released amount of mercury could be three times higher in comparison to the global background level.

In COHIBA study, all countries analysed their own samples for mercury, only German and Finnish samples were analysed by IVL laboratory.

Analytical method for effluents, storm waters and landfill leachate

Analytical methods for mercury determination in effluents, storm waters and landfill leachate samples varied among laboratories. All methods were based on cold vapour technique. The list of analytical and sample pre-treatment methods, equipment and LOQ used by countries are shown in Table 4.8.

Three different methods were used for mercury detection Cold Vapour Atomic Fluorescence Spectrometry (CV AFS), Cold Vapour Atomic Absorption Spectrometry (CV AAS) and Zeeman Atomic Absorption Spectroscopy with Modulation High Polarisation of Light (ZAAS-HFM).

Limit of quantification (LOQ) varied between laboratories (Table 4.18). It might cause the difference between findings. In some countries all results were recorded below the limit of quantification.

The annual average EQS of mercury and its compounds in water is 0.05 µg/l, maximum allowable concentration EQS – 0.07 µg/l. According to LOQ of analytical methods used by countries it looks that CV AAS method are not suitable for determination of mercury concentration in water samples. All methods of analysis applied have to be based on an uncertainty of measurement of 50 % or below ($k = 2$) estimated at the level of relevant environmental quality standards and a limit of quantification equal or below a value of 30 % of the relevant environmental quality standards.

Table 4.8. Analytical and pre-treatment method, equipment and LOQ used by countries.

Country	Detection Analytical method	Pre-treatment Equipment	LOQ
Denmark	CV AFS Based on EN 1483. Water quality. Determination of mercury. Method using atomic absorption spectrometry.	Sample digestion with mixture of bromide/bromate or analyzed directly after conservation. Sample autoclaving with KMnO_4 . Mercury was reduced to the elemental form by SnCl_2 . Analytic Jena, Mercur	5 ng/L 0.2 ng/L with gold trap
Estonia	CV AAS EVS EN 1483. Water quality. Determination of mercury. Method using atomic absorption spectrometry.	Sample digestion with $\text{HNO}_3 + \text{H}_2\text{SO}_4 + \text{KMnO}_4$ in water bath. Mercury was reduced to the elemental form by SnCl_2 . Nippon Jarrell Ash Co Ltd. Mercury detector AMD-F2	0.05 µg/l
Finland Germany Sweden	CV AFS Dual gold amalgamation and detection by CV AFS.	Samples treated with BrCl to oxidise all mercury to form Hg(II) . Mercury was reduced to the elemental form by SnCl_2 . Tekran, Model 2500 for mercury detection.	0.1 ng/l
Latvia	CV AAS US EPA 7470A. Mercury in liquid waste (manual cold-vapor technique).	Sample digestion with $\text{HNO}_3 + \text{H}_2\text{SO}_4 + \text{KMnO}_4 + \text{K}_2\text{S}_2\text{O}_8$ in water bath. Mercury was reduced to the elemental form by SnCl_2 . Varian SpectrAA 880 with cold vapour generator accessory VGA 77	0.5 µg/l
Lithuania	CV AFS LST EN ISO 17852:2008. Water quality. Determination of mercury. Method using atomic fluorescence spectrometry	Sample digestion with HNO_3 in microwave oven and additional HCl and KBrO_3/KBr . Analytic Jena, Mercur.	0.020 µg/l
Poland	ZAAS-HFM EN 12338:1998. Determination of Hg. Enrichment methods by amalgamation.	Sample digestion with $\text{HNO}_3 + \text{H}_2\text{SO}_4 + \text{KMnO}_4 + \text{K}_2\text{S}_2\text{O}_8$ in water bath. Mercury was reduced to the elemental form by SnCl_2 . LUMEX Ltd. RA-915+ with RP-91 attachment.	0.010 µg/l

Municipal waste water treatment plant

Altogether 114 municipal effluents were collected for analysis of mercury. Mercury was found in 78, i.e. 68% of municipal effluent samples (Table 4.9.).

Table 4.9. Number of samples and frequencies of mercury concentrations above LOQ in municipal effluents.

	Number of samples	Frequency
Germany	12	10
Denmark	6	4*
Estonia	12	2
Finland	18	18
Lithuania	12	1
Latvia	12	1
Poland	18	18
Sweden	24	24
TOTAL	114	78
%		68

* Danish results are shown over LOD

The concentrations of mercury higher than LOQ were detected in the municipal effluent of all countries. The maximum level 0.94 $\mu\text{g/l}$ was observed in the municipal effluent sampled in Denmark. High concentration of mercury was detected in municipal effluent sampled in Latvia (Figure 4.6.).

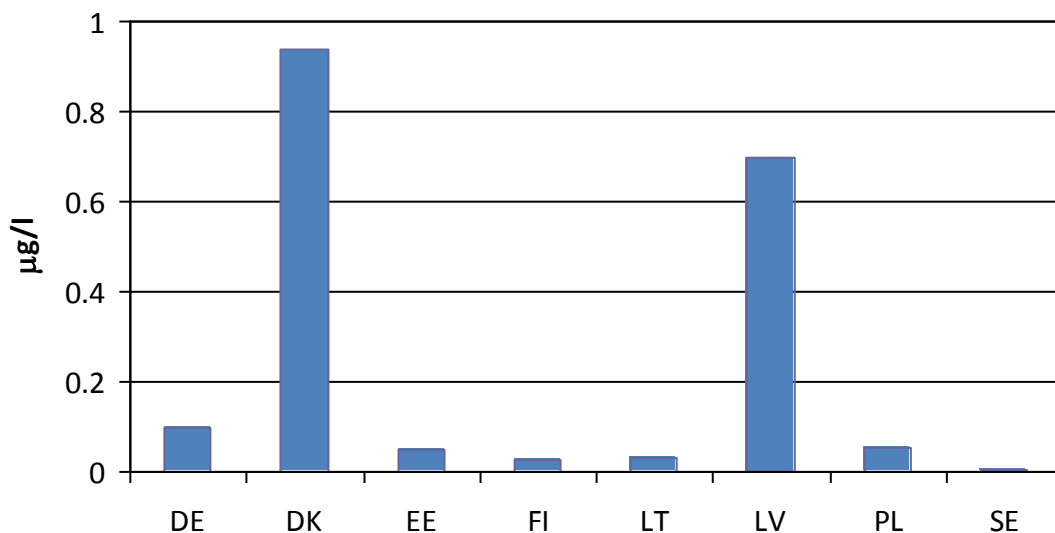


Figure 4.6. Maximum concentrations ($\mu\text{g/l}$) of mercury found in municipal effluents. One German sample (MWWTP1) was excluded because of contamination during sampling process.

Industrial waste water treatment plant

In total 57 industrial effluents were collected for analysis of Mercury. Mercury was found in 26, i.e. 46% of industrial effluent samples (Table 4.10.).

Table 4.10. Number of samples and frequencies of Hg concentrations above LOQ in industrial effluents.

	Number of samples	Frequency
Germany	11	8
Denmark	4	0*
Estonia	12	1
Finland	6	6
Lithuania	6	5
Latvia	12	0
Poland	6	6
Sweden	0	0
TOTAL	57	26
%		46

* Danish results are shown over LOD

The concentrations of mercury higher than LOQ were detected in the industrial effluent of five countries (Figure 4.7.). The maximum level 4.4 $\mu\text{g/l}$ was observed in the industrial effluent sampled in Germany. There were no industrial effluent samples taken from Sweden, where all of the wastewater treatment plants were considered to be municipal.

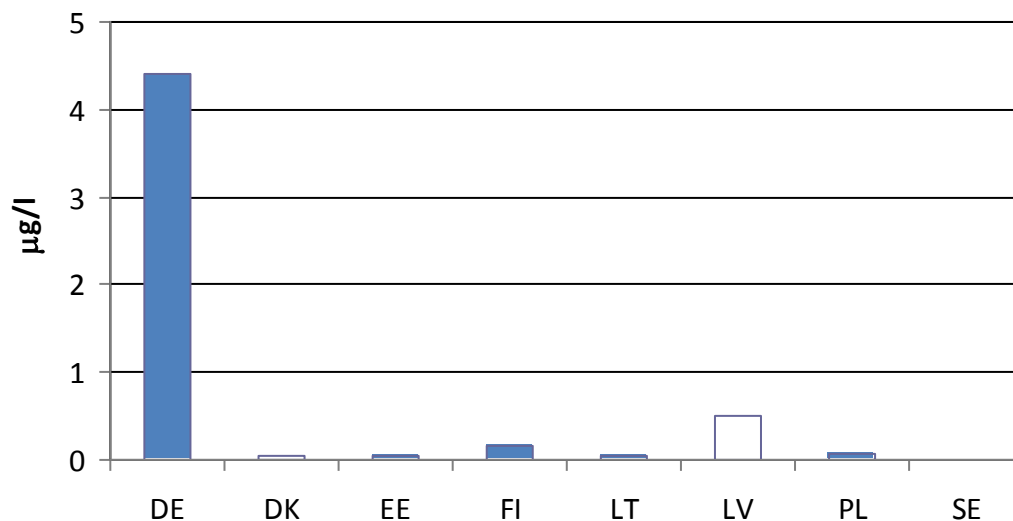


Figure 4.7. Maximum concentrations ($\mu\text{g/l}$) of mercury found in industrial effluents. When the maximum concentration was found under the LOQ, the bar is left blank.

Storm water

In total 15 storm water were collected for analysis of Mercury. Mercury was found in 11, i.e. 73% of storm waters (Table 4.11.).

Table 4.11. Number of samples and frequencies of mercury concentrations above LOQ in storm waters.

	Number of samples	Frequency
Germany	2	2
Denmark	2	2*
Estonia	2	0
Finland	2	2
Lithuania	2	1
Latvia	1	0**
Poland	2	2
Sweden	2	2
TOTAL	15	11
%		73

*Danish results are shown over LOD

** Latvian results below LOD

The concentrations of mercury higher than LOQ were detected in the storm waters of six countries (Figure 4.8.). The maximum level 0.29 $\mu\text{g/l}$ was observed in the storm waters sampled in Denmark. It should be noted that the maximum level of concentration in the storm water sampled in Denmark was lower than LOQ but higher than LOD for storm water sampled and analysed in Latvia.

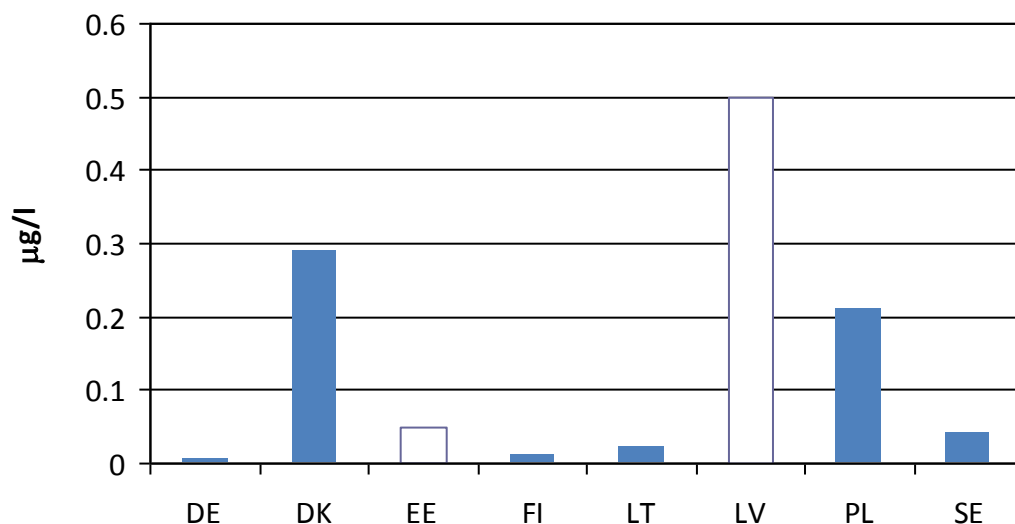


Figure 4.8. Maximum concentrations ($\mu\text{g/l}$) of mercury found in storm waters. In case where the maximum concentration was found under the LOQ, the bar is left blank.

Landfill leachate

In total 16 landfill leachate samples were collected for analysis of Mercury. Mercury was found in 10, i.e. 62% of landfill leachate samples (Table 4.12.).

Table 4.12. Number of samples and frequencies of mercury concentrations above LOQ in landfill leachates.

	Number of samples	Frequency
Germany	2	2
Denmark	2	1*
Estonia	2	1
Finland	2	2
Lithuania	2	0
Latvia	2	0
Poland	2	2
Sweden	2	2
TOTAL	16	10
%		62

*Danish results are shown over LOD

The concentrations of mercury higher than LOQ were detected in the landfill leachate of six countries. The maximum level 0.47 µg/l was observed in the landfill leachate sampled in Poland (Figure 4.9.). It should be noted that the maximum level of concentration in the landfill leachate sampled in Poland was lower than LOQ for landfill leachate sampled and analysed in Latvia.

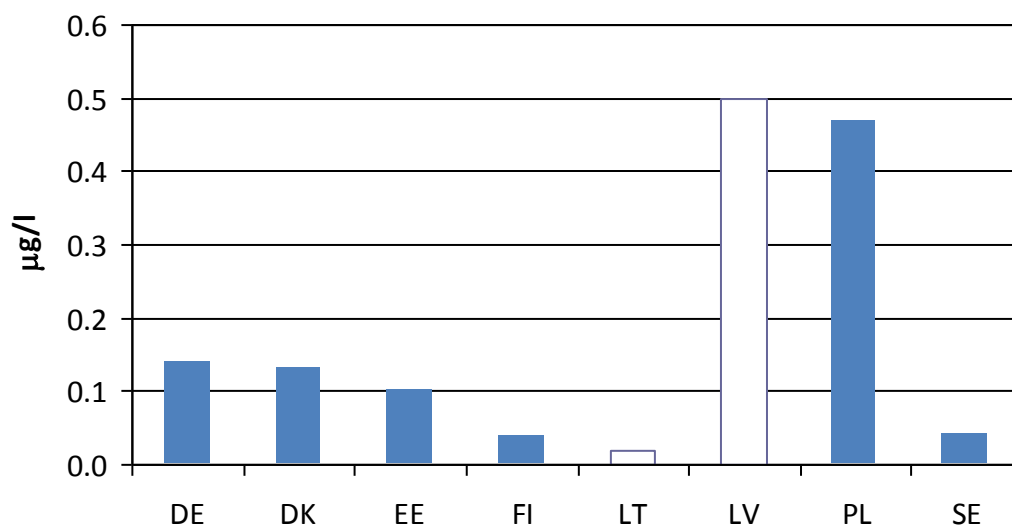


Figure 4.9. Maximum concentrations (µg/l) of mercury found in landfill leachates. When maximum concentration was found under the LOQ, the bar is left blank.

Analytical methods for sludge

The analytical methods for sludge samples varied between laboratories. All methods were based on cold vapour technique. The list of analytical and sample pre-treatment methods, equipment and LOQ used by countries are shown in Table 4.13.

Three different methods were used for mercury detection Cold Vapour Atomic Fluorescence Spectrometry (CV AFS), Cold Vapour Atomic Absorption Spectrometry (CV AAS) and Zeeman Atomic Absorption Spectroscopy with Modulation High Polarisation of Light (ZAAS-HFM).

Table 4.13. Analytical and pre-treatment method, equipment and LOQ (mg/kg) used by countries in sludge. There was no information about LOQ for Danish laboratories and there were no sludge samples from Latvia.

Country	Detection Analytical method	Pre-treatment Equipment	LOQ
Denmark	CV AAS Based on <i>EN 1483</i> . Water quality. Determination of mercury. Method using atomic absorption spectrometry.	Sample autoclaving with $\text{HNO}_3 + \text{H}_2\text{SO}_4 + \text{KMnO}_4$. Mercury was reduced to the elemental form by SnCl_2 . Analytic Jena, Mercur	
Estonia	CV AAS <i>EVS EN 1483</i> . Water quality. Determination of mercury. Method using atomic absorption spectrometry.	Sample digestion with $\text{HNO}_3 + \text{H}_2\text{SO}_4 + \text{KMnO}_4$ in water bath. Mercury was reduced to the elemental form by SnCl_2 . Nippon Jarrell Ash Co Ltd. Mercury detector AMD-F2	0.02
Finland Germany Sweden	CV AFS Dual gold amalgamation and detection by CV AFS.	Samples were digested $\text{HCl} + \text{HNO}_3$, treated with BrCl to oxidise all mercury to form Hg(II) . Mercury was reduced to the elemental form by SnCl_2 . Tekran, Model 2500 for mercury detection.	0.0001
Latvia			
Lithuania	CV AFS <i>LST EN ISO 17852:2008</i> . Water quality. Determination of mercury. Method using atomic fluorescence spectrometry	Sample digestion with HNO_3 in microwave oven and additional digestion $\text{HCl} + \text{KBrO}_3/\text{KBr}$. Analytic Jena, Mercur.	0.050
Poland	ZAAS-HFM <i>EN 12338:1998</i> . Water quality. Determination of mercury. Enrichment methods by amalgamation.	Sample digestion with $\text{HCl} + \text{HNO}_3$. Mercury was reduced to the elemental form by SnCl_2 . LUMEX Ltd. RA-915+ with RP-91 attachment.	0.010

Sludge samples

In total 18 sludge samples were collected for analysis of mercury. Mercury was found in all samples, i.e. 100% of landfill leachate samples (Table 4.14.).

Table 4.14. Number of samples and observed frequencies of mercury concentrations above LOQ in sludge.

	Number of samples	Frequency
Germany	3	3
Denmark	4	4*
Estonia	3	3
Finland	2	2
Lithuania	2	2
Latvia	0	0
Poland	2	2
Sweden	2	2
TOTAL	18	18
%		100

* LOQ for Danish results are not known

The concentrations of mercury higher than LOQ were detected in all samples analysed by countries. The maximum level 2.7 mg/kg was observed in the sewage sludge sampled in Denmark (Figure 4.10.). There were no sludge samples taken from Latvia.

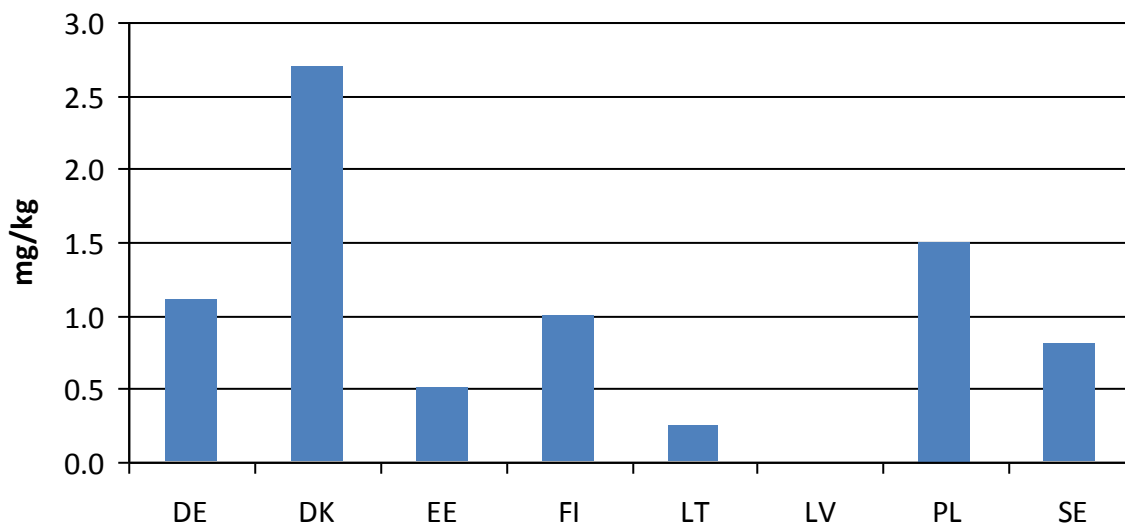


Figure 4.10. Maximum concentrations (mg/kg) of mercury found in sludge samples.

4.2 Organotins

Organotins are broad-spectrum biocides with persistent, accumulating, toxic and endocrine disrupting abilities. Especially crayfish and mollusks e.g. snails and mussels are vulnerable towards tributyltin (TBT) and triphenyltin (TPhT). Concentrations of less than 1 ng/l TBT can inhibit growth and disturb reproduction, the immune system and/or the survival rate. Di- and mono-butyltins (DBT and MBT) are in general less toxic than TBT and TPhT.

Monobutyltins and monooctyltins (MOT) are used as PVC heat stabilizers. Diphenyltins are used in polymer manufacturing, in the manufacturing of polyurethane and silicone curing. TBTs are used as industrial biocides, e.g. as antifungal agents in textiles and paper, wood pulp and paper mill systems. TBTs have also been used in marine anti-fouling agent, but concern over their toxicity has led to a worldwide ban by the International Maritime Organization. Triphenyltins are used as active components of antifungal paints and agricultural fungicides.

Analytical methods

IVL (Swedish Environmental Research Institute) analysed organotins in the samples from all countries except Denmark, who analysed their own samples. The compounds analysed at IVL were monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), tetrabutyltin (TeBT, not analyzed in sludge), triphenyltin (TPhT), monooctyltin (MOT), dioctyltin (DOT) and tricyclohexyltin (TCT). At IVL water samples were simultaneously ethylated and extracted followed by analysis using GC-MS-MS (SS-EN ISO 17353:2005 mod). Instruments used were 7890A GC interfaced to 7000A Triple Quad MS (Agilent). Acidic extraction of freeze dried sludge was followed by ethylation and analysis using GC-MS-MS (ISO 23161:2009 mod). Instruments used were the same as for water samples. LOQ for water samples was 0.001 µg/l (0.001-0.01 µg/l for storm-water), while the LOQ for sludge was 1–5 µg/kg dw (dry weight). The Danish samples were analysed by Eurofins Environment (Denmark). In the Danish samples the following organotins were analysed: MBT, DBT, TBT and TPhT. Water samples were derivatized in the aqueous phase with sodium borate tetra ethylene, after which tetraalkyltin relations were extracted from the aqueous phase with pentane. The extract was concentrated by rotary evaporation and analyzed by capillary GC-MS. Quantification was performed with samples of tap water containing a known amount of analyte, and incidentally generated and analyzed as real samples. The analysis was performed by gas chromatography with mass spectrometric detector using selective ion monitoring (SIM). LOQ for waste waters was 0.001-0.01 µg/l.

Results

Municipal waste water treatment plant (MWWTP)

Number of samples and observed frequencies of organotin compounds in effluents from MWWTP are listed in Table 4.15.

Table 4.15. Number of samples and observed frequencies of organotin compounds observed above LOQ in effluents from municipal treatment plants.

	No of samples	MBT	DBT	TBT	TeBT	TPhT	MOT	DOT	TCT
Denmark	6	1	0	0	na	0	na	na	na
Estonia	12	6	5	2	1	0	3	1	0
Finland	18	13	13	1	0	0	0	1	0
Germany	12	9	9	1	0	0	1	0	0
Latvia	4	4	3	0	0	0	0	0	0
Lithuania	12	6	6	0	0	0	0	0	0
Poland	18	14	10	3	1	1	9	3	0
Sweden	24	10	7	0	0	0	1	0	0
TOTAL	106	63	53	7	2	1	14	5	0
%		59	50	7	2	1	13	5	0

TPhT was only found in one sample (Poland, 1.1 ng/l), and TCT was not found in any sample, at concentrations higher than LOQ. For the other organotins, the maximum concentrations in the different countries are summarised in figure 4.11. MBT was found in 59 % of the effluent samples and in all countries, with concentrations > 15 ng/l found in Finnish, German and Lithuanian samples. DBT was also frequently found (50% of samples) except in Denmark (maybe due to different LOQ). MOT was found occasionally (13 % of samples) in four of the eight countries, most frequently and with highest concentrations (up to 9.4 ng/l) in Polish samples. The other analysed compounds were found in < 10 % of the samples.

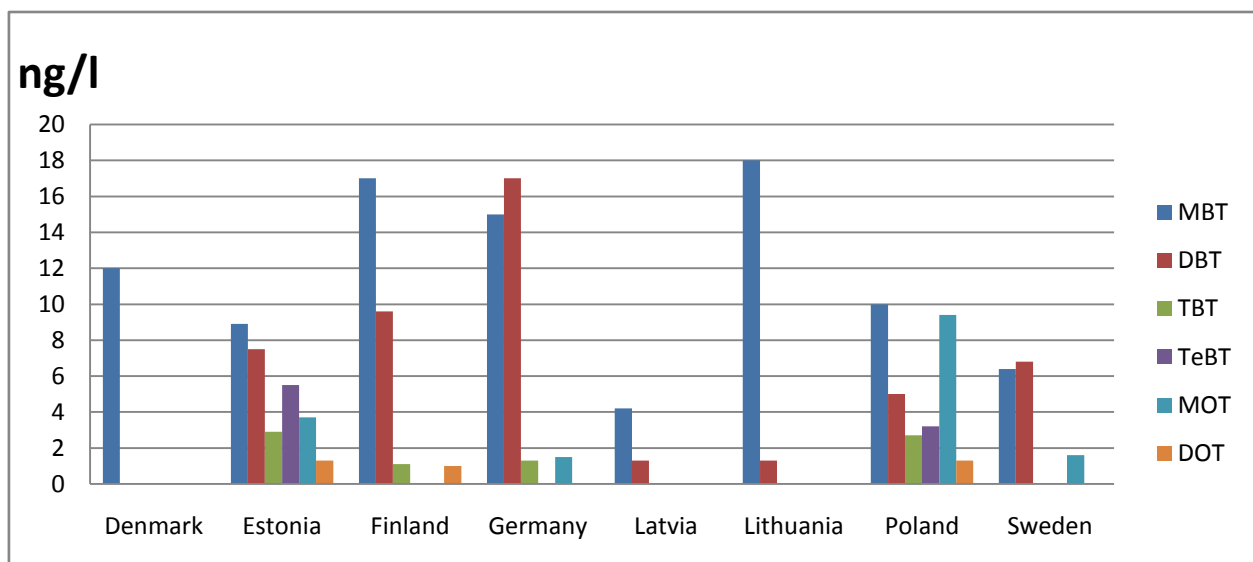


Figure 4.11. Maximum concentrations (ng/l) of organotin compounds found in municipal effluents.

TBT was found in 7 % of the samples with highest concentrations (2-3 ng/l) in Estonian and Polish effluents. A European EQS is set for TBT at 0.2 ng/l. All detected concentrations as well as LOQ is above the EQS for TBT.

MBT, DBT, TBT, MOT and DOT were detected in 79% of the sludge samples from MWWTPs. In sludge from Danish MWWTPs there were no organotin compounds found above their LOQ. There were no sludge samples from Estonia. TeBT was not analysed in any sludge samples (Table 4.16).

Table 4.16. Number of samples and observed frequencies of organotin compounds observed above LOQ in sludge from municipal treatment plants (na, not analysed).

	Number of samples	MBT	DBT	TBT	TPhT	MOT	DOT	TCT
Denmark	4	0	0	0	0	na	na	na
Finland	2	2	2	2	0	2	2	0
Germany	3	3	3	3	0	3	3	0
Latvia	4	4	4	4	4	4	4	3
Lithuania	2	2	2	2	0	2	2	1
Poland	2	2	2	2	1	2	2	1
Sweden	2	2	2	2	0	2	2	0
TOTAL	19	15	15	15	5	15	15	5
%		79	79	79	26	79	79	26

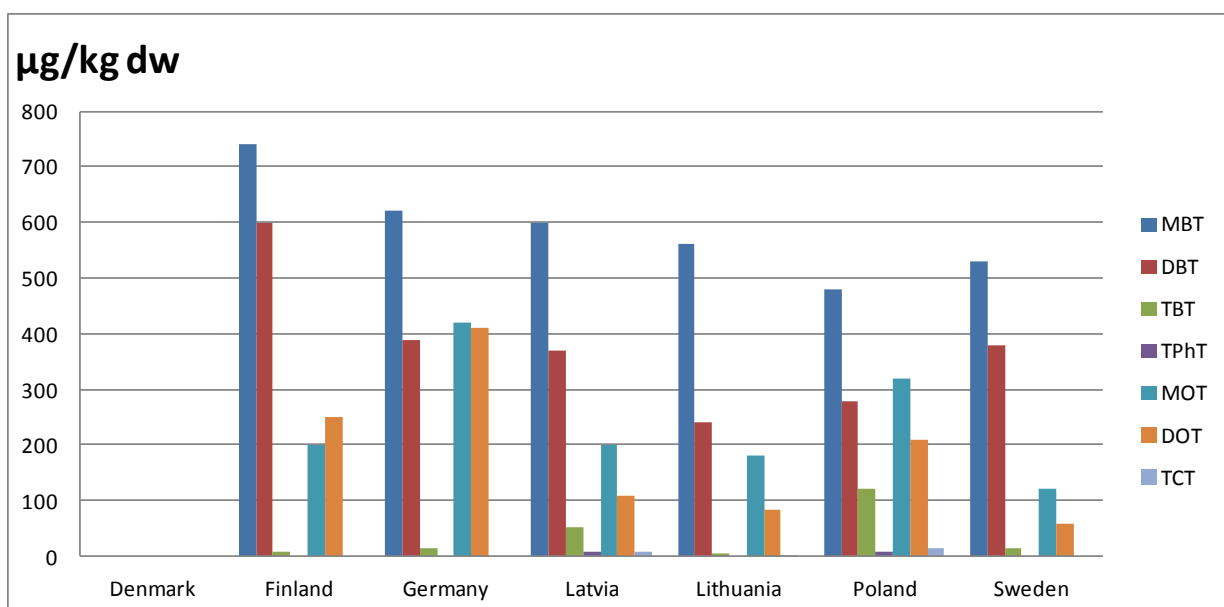


Figure 4.12. Maximum concentrations ($\mu\text{g}/\text{kg}$ dry weight) of organotin compounds found in sludge from municipal WWTP.

The maximum concentrations detected in MWWTP sludge in the different countries are summarised in figure 4.13. MBT was found at highest concentrations in all countries. However, sludge data compared to effluent data indicates that DBT, TBT, MOT and DOT accumulate more efficiently in sludge compared to MBT.

Industrial waste water treatment plant

There were no samples from industrial WWTP in Sweden. For the other countries the number of samples and frequencies of concentrations above LOQ is reported in table 4.17.

MBT was also here most frequently found. TPhT and TCT were not found in any sample at concentrations higher than LOQ while TBT and DOT only were found in one sample each.

Table 4.17. Number of samples and observed frequencies of organotin compounds observed above LOQ in effluents from industrial treatment plants.

	Number of samples	MBT	DBT	TBT	TeBT	TPhT	MOT	DOT	TCT
Denmark	4	2	2	1	na	0	na	na	na
Estonia	12	9	5	0	2	0	5	0	0
Finland	6	3	4	0	0	0	0	0	0
Germany	11	8	5	0	0	0	1	0	0
Latvia	3	1	1	0	0	0	0	0	0
Lithuania	12	3	1	0	0	0	0	0	0
Poland	6	1	3	0	1	0	1	1	0
Sweden	0								
TOTAL	54	27	21	1	3	0	7	1	0
%		50	39	2	6	0	13	2	0

The kind of industry sites included in the study vary considerably between countries which is indicated by the differing concentration patterns in the different countries (Figure 4.13.). The only measurable TBT concentration, as high as 110 ng/l, was in runoff water from a shredder plant in Denmark while the only DOT value, 4.9 ng/l, was from the Polish industry site (activity not defined). MBT and DBT were most frequently found (50 and 39 % of samples respectively). Highest concentrations (about 100 ng/l) were found for TBT (Denmark), MOT and DOT (Poland), but these were one occasion measurements and are therefore difficult to draw any conclusions from.

Sludge from industrial WWTP was only sampled in Estonia. Highest concentrations were reported for MBT and DBT, 420 and 230 µg/kg dw respectively. Medium concentrations were reported for MOT and DOT, 83 and 35 µg/kg dw respectively, while low concentrations were found for TBT and TCT, 7.4 and 1.9 µg/kg dw respectively.

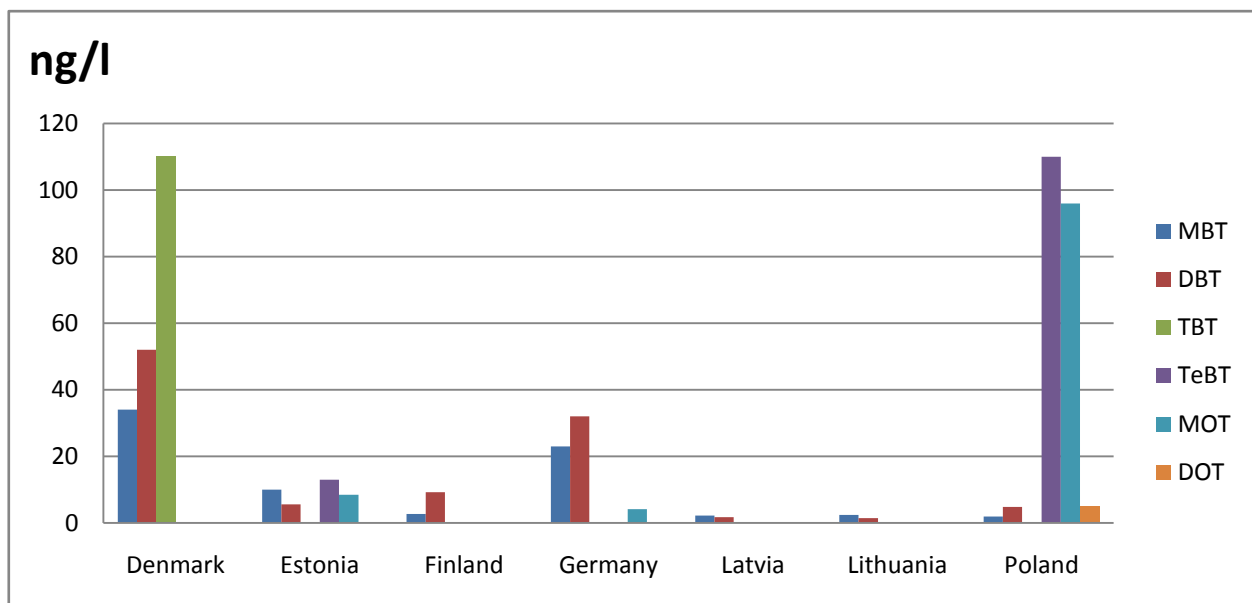


Figure 4.13. Maximum concentrations (ng/l) of organotin compounds measured in industrial effluents.

Storm water

The few storm-water analyses indicate most frequent occurrence for MBT and DBT, as was the case in municipal as well as industrial WWTP effluents, while TBT was found at higher frequency compared to WWTP effluents. TeBT, TPhT and TCT were not detected above LOQ in any sample (Table 4.18.). No organotins above LOQ were detected in the two samples from Lithuania.

Table 4.18. Number of samples and observed frequencies of organotin compounds observed above LOQ in stormwater.

	Number of samples	MBT	DBT	TBT	TeBT	TPhT	MOT	DOT	TCT
Denmark	2	2	2	0	na	0	na	na	na
Estonia	2	0	0	2	0	0	1	0	0
Finland	2	1	1	0	0	0	0	0	0
Germany	2	1	1	0	0	0	0	0	0
Latvia	1	0	1	1	0	0	0	1	0
Lithuania	2	0	0	0	0	0	0	0	0
Poland	2	0	0	1	0	0	0	0	0
Sweden	2	1	1	0	0	0	1	1	0
TOTAL	15	5	6	4	0	0	2	2	0
%		33	40	27	0	0	13	13	0

It is not possible to draw firm conclusions regarding differences in concentrations (Figure 4.14.) based on the very few data, although the data indicate that MBT was not as dominating as in the MWWTP effluents.

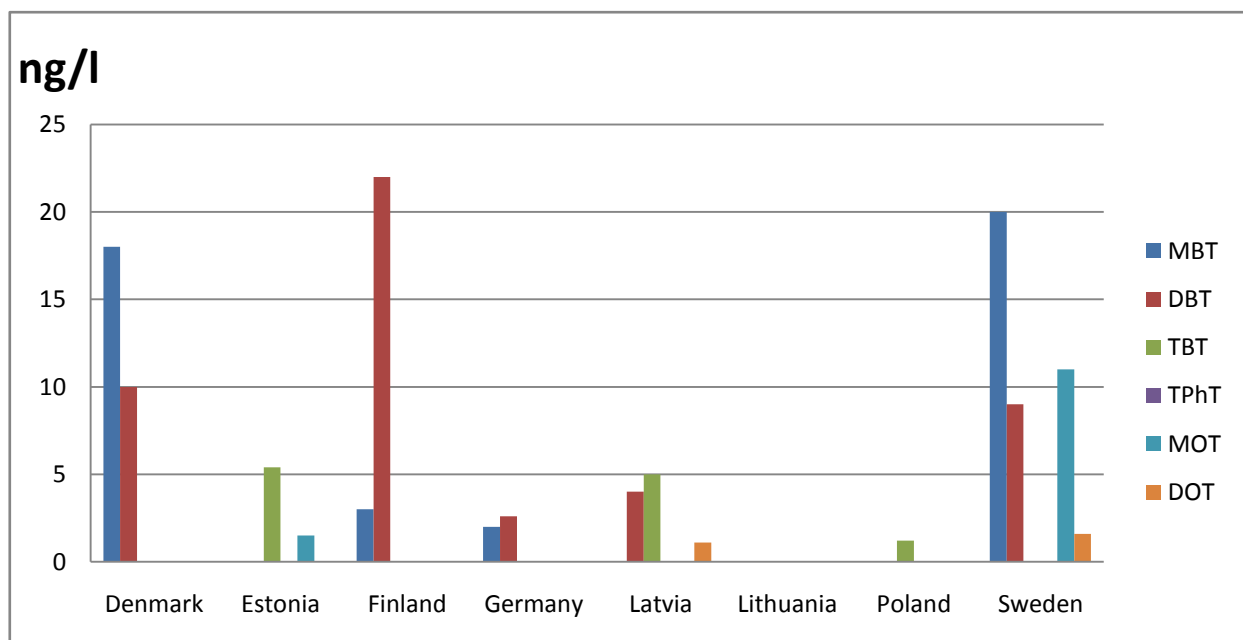


Figure 4.14. Maximum concentrations (ng/l) of organotin compounds measured in storm water.

Landfill leachate

In leachate from landfills MBT dominated, in occurrence (Table 4.19.) as well as concentrations (Figure 4.15.). TPhT and TCT were not detected in any sample.

Table 4.19. Number of samples and observed frequencies of organotin compounds observed above LOQ in landfill leachate.

	Number of samples	MBT	DBT	TBT	TeBT	TPhT	MOT	DOT	TCT
Denmark	2	1	0	0	na	0	na	na	na
Estonia	2	2	0	0	0	0	1	0	0
Finland	2	1	1	1	0	0	1	0	0
Germany	2	0	1	0	0	0	0	0	0
Latvia	1	1	0	0	0	0	0	0	0
Lithuania	2	1	0	0	0	0	1	0	0
Poland	2	2	2	2	2	0	2	2	0
Sweden	2	1	0	0	0	0	0	0	0
TOTAL	15	9	4	3	2	0	5	2	0
%		60	26	20	13	0	33	13	0

The measured maximum concentrations in the samples from Poland were an order of magnitude higher than in the other countries (note the logarithmic scale in figure 4.15.). The maximum MBT and TBT concentration in the Polish samples were 780 ng/l and 28 ng/l respectively. The Polish landfill was used from 1977 to 2007. Currently, the landfill is at a post-operation stage. The amount of deposited sludge is estimated at about 2.5 million tons. The landfill leachate is collected and pumped to the municipal waste water treatment plant.

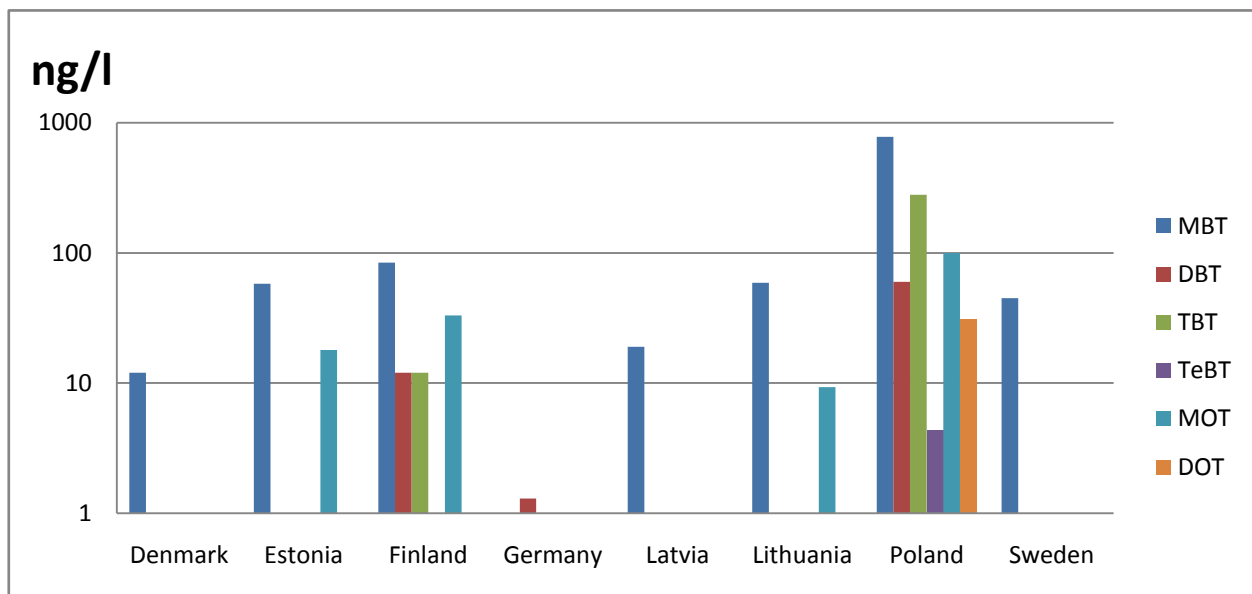


Figure 4.15. Maximum concentrations (ng/l) of organotin compounds measured in landfill leachate.

Concentrations in the Baltic Sea

In Denmark, measurements of concentrations at different sites in the Baltic Sea were also carried out. The highest concentrations of MBT were found in the Baltic Sea, 130 ng/l, and in one of the two samples from the Sound, 230 ng/l. These concentrations are higher than most concentrations in effluents reported above. The other sample from the Sound did not show a concentration of MBT above the detection limit (10 ng/l). DBT and TBT were not detected above the detection limit in the samples from the Baltic Sea and the Sound. The Danish concluded that the high concentrations of MBT probably are a result of the degradation of TBT due to the earlier use of TBT in antifouling paints for ships.

4.3 Phenolic substances

4-Nonylphenols are a number of isomeric phenol compounds with normal or branched hydrocarbon chain. Nonylphenol (4-NP) is used in *e.g.* paint, plastic industry and in the production of washing agents and nonylphenol ethoxylates. Nonylphenol ethoxylates are mixtures of nonionic surfactants used as detergents, emulsifiers, wetting agents, defoaming agents, etc. Nonylphenol and nonylphenol ethoxylates have been banned in the European Union as hazard to human and environmental safety.

Octylphenols (OP) are mainly used for the production of phenol resins, which are used in rubber production as a tackifier for tyres. Octylphenol ethoxylates are used for many purposes, mainly for the production of cleaning products such as detergents, but also in lesser quantities in many other applications, such as pesticides, paints and varnishes. Octylphenol is toxic for fishes and shows endocrine effects.

Nonylphenol ethoxylates and bisphenol A (BPA) are known as endocrine disruptors, which can mimic the body's own hormones and thus lead to negative health effects. BPA especially, can affect growth, reproduction and development of aquatic organisms.

SYKE analysed phenolic substances also for Estonia, Germany, Latvia, Lithuania and Poland. IVL analysed Swedish samples and Eurofins Danish samples. In this study bisphenol A (BPA), 4-nonylphenol (4-NP), 4-nonylphenol monoethoxylate (4-NP1EO), 4-nonylphenol diethoxylate (4-NP2EO), octylphenol (OP), octylphenol monoethoxylate (4-OP1EO), octylphenol diethoxylate (4-OP2EO) were analysed.

Analytical method for effluents, storm waters and landfill leachates

The analytical methods for effluents, storm waters and landfill leachates varied between laboratories. In Finland (SYKE) the internal standards ($^{13}\text{C}_6$ -ring 4-NP, $^{13}\text{C}_6$ -ring 4-NP1EO, $^{13}\text{C}_6$ -ring NP2EO, $^{13}\text{C}_6$ -ring OP, $^{13}\text{C}_6$ -ring OP1EO, $^{13}\text{C}_6$ -ring OP2EO and d_{16} BPA) were added to acidified (pH <3) samples before extraction. The 100 ml of whole water sample was extracted and purified with acetone-methanol- H_2O -conditioned solid phase extraction disks using vacuum. The compounds were eluted from the cartridges with acetone without vacuum. Elute was evaporated to dryness by N_2 stream (30°C). 1 ml methanol/water (v/v, 1/1) was used to re-dissolve the sample and the recovery standard (pentyphenol) was added to the sample vials. LC-MS (ESI) was used for instrumental analysis of the compounds. The blank sample was determined in all sample series.

In Sweden (IVL) acidified sample was purified and concentrated by SPE (Isolut ENV+ 200 mg) eluting with acetonitrile, tetrahydrofuran, hexane + MTBE (1+1). Organic phase was reduced in volume, dried, acetylated (acetic anhydride, sodium acetate) and cleaned on silica column (5% water).

In Denmark (Eurofins) samples were extracted with cyclohexane, derivatised and analysed by GC-MS.

In all sample types LOQs varied between laboratories and substances. In some countries all observations above the detection limit (DL) were recorded and for the congruence, DL was used in frequency studies.

In the ring-test, the results of two of the laboratories for the standard solutions were congruent. The BPA results from landfill leachate were not comparable. However, the results for 4-NP, 4-NP1EO, 4-NP2EO, OP1EO and OP2EO in landfill leachate were quite similar between two of the laboratories. The variation in OP results is partly caused by different LOQs. The results of municipal effluent differed, but they were in the same level (close to LOQ).

Municipal effluent results

In total, 106 municipal effluents were collected for analysis of phenolic compounds. Bisphenol A was found in 67 % of municipal effluent samples. The maximum concentration (5.8 µg/l) was observed in Estonian effluent (Figure 4.16.).

In the case of municipal effluent 4-nonylphenol, 4-nonylphenol monoethoxylate and 4-nonylphenol diethoxylate were observed in 79 %, 49% and 42% of taken samples, respectively. The maximum concentrations were found for 4-nonylphenol (2.2 µg/l) in Germany, for 4-nonylphenol monoethoxylate (0.5 µg/l) in Poland and for 4-nonylphenol diethoxylate (0.19 µg/l) in Estonia.

Octylphenol, octylphenol monoethoxylate and octylphenol diethoxylate were found in 54%, 20% and 5% of municipal effluent samples, respectively. The maximum concentrations were noted for octylphenol (0.32 µg/l) in Poland and for octylphenol mono- and diethoxylates (0.51 and 0.24 µg/l, respectively) in Sweden.

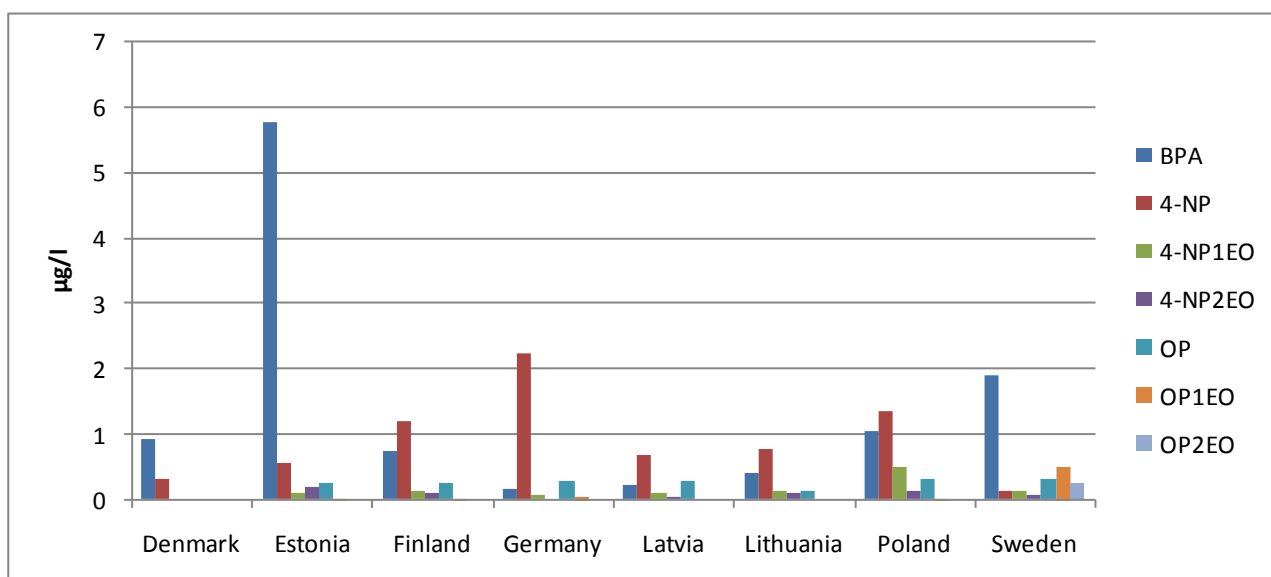


Figure 4.16. Maximum concentrations (µg/l) of phenolic compounds found in municipal effluents.

In municipal effluents the most frequently found phenolic substances were 4-nonylphenol, bisphenol A and octylphenol. Bisphenol A and 4-nonylphenol were found in every country and octylphenol diethoxylate was found only in Sweden. In municipal effluents the highest concentrations were observed for bisphenol A and 4-nonylphenol.

Industrial waste water treatment plant

In total 55 industrial effluents were analysed of phenolic compounds. In 49 % of industrial effluent samples bisphenol A was observed. The maximum concentration (92 µg/l) was noted in Denmark (Figure 4.17.), but it has to be noted that there is no treatment of the industrial wastewater except sedimentation and pH-neutralisation, where this concentration was measured.

In industrial effluent 4-nonylphenol, 4-nonylphenol monoethoxylate and 4-nonylphenol diethoxylate were found in 78 %, 40% and 29% of samples, respectively. The maximum concentrations were noted for 4-nonylphenol (2.6 µg/l), 4-nonylphenol monoethoxylate (6.4 µg/l) and 4-nonylphenol diethoxylate (7.0 µg/l) in Estonia.

Octylphenol, octylphenol monoethoxylate and octylphenol diethoxylate were observed in 38%, 18% and 11% of industrial effluent samples, respectively. The maximum concentrations were found for octylphenol (0.36 µg/l) in Latvia and for octylphenol mono- and diethoxylates (3.8 µg/l and 25 µg/l, respectively) in Denmark.

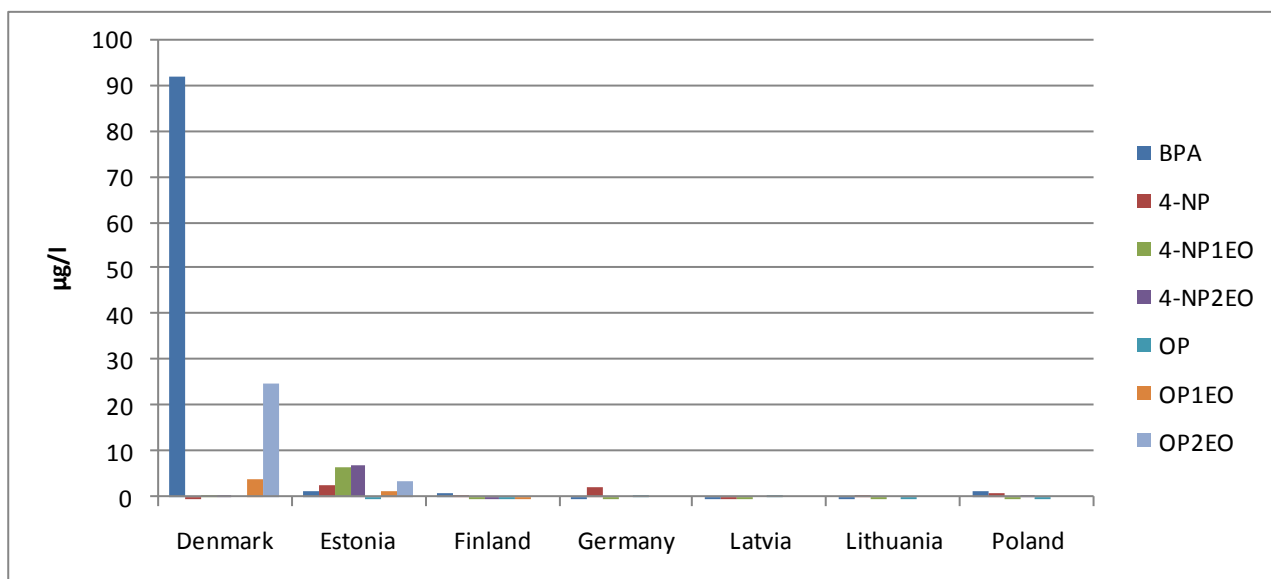


Figure 4.17. Maximum concentrations (µg/l) of phenolic compounds found in industrial effluents.

In industrial effluent most frequently found phenolic substances were 4-nonylphenol and bisphenol A. Bisphenol A, 4-nonylphenol and 4-nonylphenol monoethoxylate were found in every country. Octylphenol diethoxylate was found only in two countries, in Denmark and Estonia.

In industrial effluent the highest concentrations were observed for bisphenol A and octylphenol diethoxylate.

Storm water

In totally 15 storm water effluents were collected for analysis of phenolic compounds. Bisphenol A was found in 93 % of storm water samples. The maximum concentration (3.1 µg/l) was found in Germany (Figure 4.18).

4-Nonylphenol, 4-nonylphenol monoethoxylate and 4-nonylphenol diethoxylate were observed in 73 %, 47% and 47% of storm water samples, respectively. The maximum concentrations were found for 4-nonylphenol (2.6 µg/l) in Latvia, 4-nonylphenol monoethoxylate (4.7 µg/l) in Sweden and for 4-nonylphenol diethoxylate (0.2 µg/l) in Latvia.

In the case of storm water octylphenol, octylphenol monoethoxylate and octylphenol diethoxylate were found in 27%, 27% and 7% of samples, respectively. The maximum concentrations were observed for octylphenol (0.24 µg/l) in Latvia and for octylphenol mono- and diethoxylate (0.24 µg/l and 0.47 µg/l, respectively) in Sweden.

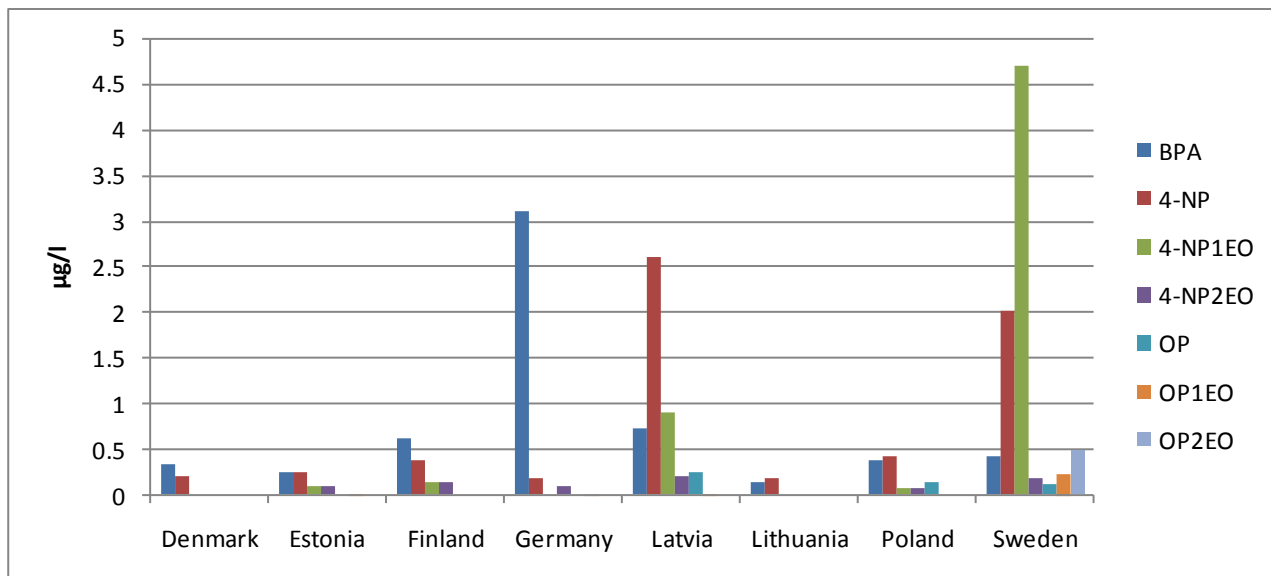


Figure 4.18. Maximum concentrations (µg/l) of phenolic compounds found in storm water.

In storm water most frequently found phenolic substances are bisphenol A and 4-nonylphenol. Bisphenol A were found in every country. Octylphenol diethoxylate was found only in Sweden.

In storm water the highest concentrations were found for 4-nonylphenol monoethoxylate and bisphenol A.

Landfill leachate

In total, 15 landfill leachate samples were collected for analysis of phenolic compounds. Bisphenol A was found in all landfill leachate samples. The maximum concentration (~700 µg/l) was observed in Poland (Figure 4.19.).

In landfill leachate 4-nonylphenol, 4-nonylphenol monoethoxylate and 4-nonylphenol diethoxylate were noted in 67 %, 20% and 40% of samples, respectively. The maximum concentrations were found for 4-NP (15 µg/l), 4-NP1EO (2.0 µg/l) and 4-NP2EO (0.2 µg/l) in Poland.

Octylphenol, octylphenol monoethoxylate and octylphenol diethoxylate were observed in 47%, 40% and 13% of landfill leachate samples, respectively. The maximum octylphenol and octylphenol diethoxylate concentration (1.0 and 0.09 µg/l, respectively) was found in Poland, octylphenol monoethoxylate (0.07 µg/l) in Germany and Finland.

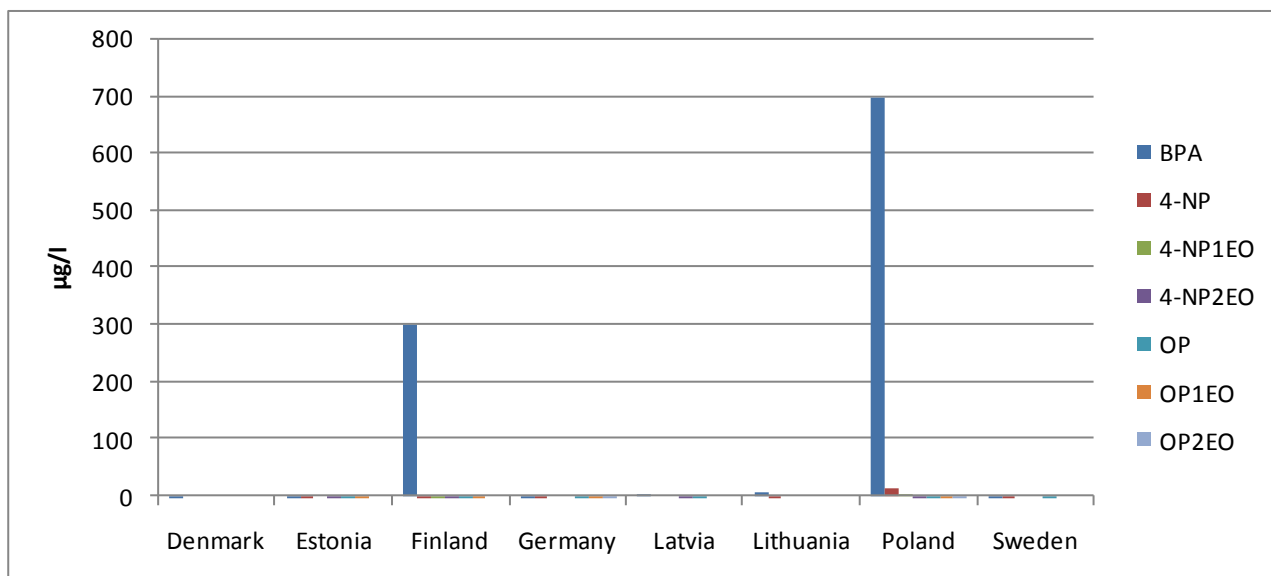


Figure 4.19. Maximum concentrations ($\mu\text{g/l}$) of phenolic compounds found in landfill leachates.

Landfill leachates taken in Finland, Denmark, Lithuania and Poland were untreated ones. In Poland and Finland the samples were taken as composite samples. All other leachates were taken as grab samples.

In landfill leachate most frequently found phenolic substances were bisphenol A and 4-nonylphenol. The highest concentrations were found for bisphenol A.

Analytical methods of sludge

In SYKE surrogate standard (^{12}C -heptylphenol) was added to the sample (2-3 g dw) before shaking with acetone-pentane (2 h). Acetone was removed by shaking with water. Separated pentane layer was evaporated just to the dryness and the sample was re-dissolved to methanol. Water (pH 2-3) was added so that the methanol volume was less than 40%. The quantification standards (^{13}C -ring 4-NP, ^{13}C -ring 4-NP1EO, ^{13}C -ring NP2EO, ^{13}C -ring OP, ^{13}C -ring OP1EO, ^{13}C -ring OP2EO and d_{16} -BPa) were added. The conditioned solid phase extraction cartridge (styrene-divinylbenzene polymer, SDB) was used for sample purification. The acetone extract was evaporated to the dryness (30°C) with nitrogen, re-dissolved with methanol/water and the injection standard (^{12}C -pentyphenol) was added.

Sludge

Altogether 22 sludge samples were analysed for phenolic compounds. In 27% of sludge samples bisphenol A was found. The maximum concentration (0.42 mg/kg) was found in Sweden (Figure 4.20.).

4-NP was detected in all sludge samples. 4-NP1EO and 4-NP2EO were observed in 86% and 77% of the sludge samples, respectively. The maximum concentrations were found for 4-NP (37 mg/kg) in Poland, for 4-NP1EO (31 mg/kg) and 4-NP2EO (26 mg/kg) in Estonia.

In sludge OP, OP1EO and OP2EO were found in 68%, 41% and 32% of samples, respectively. The maximum concentrations were noted for octylphenol (1.3 mg/kg) in Finland and for octylphenol mono- and diethoxylate (5.1 mg/kg and 9.6 mg/kg, respectively) in Estonia.

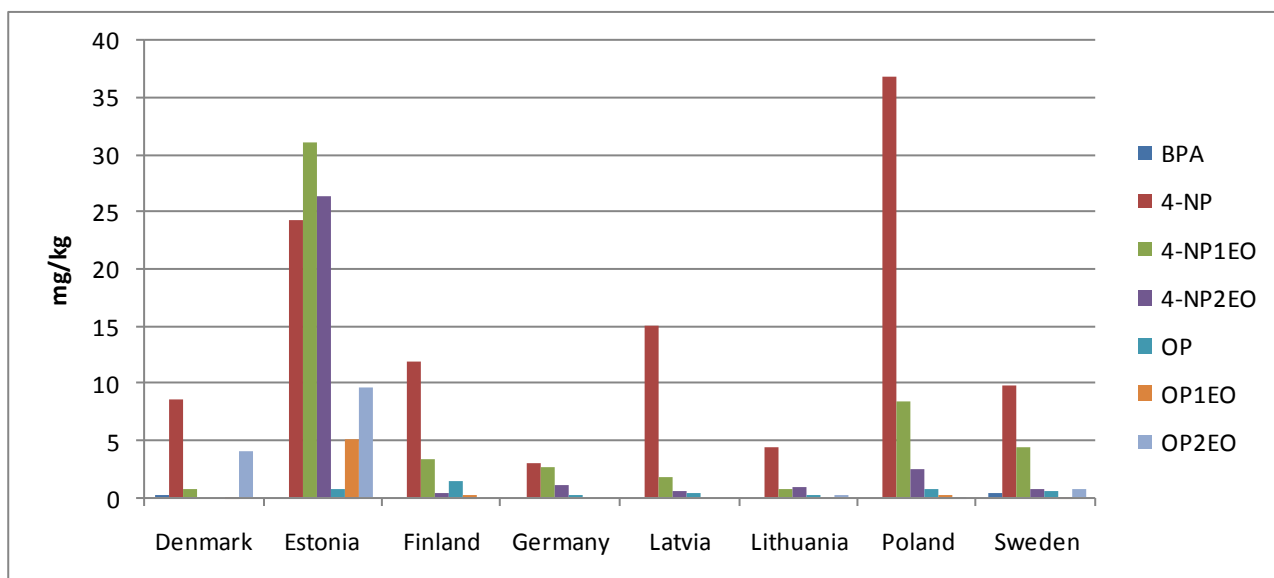


Figure 4.20. Maximum concentrations (mg/kg) of phenolic compounds found in sludge.

The most frequently found phenolic substances in sludge were 4-nonylphenol and 4-nonylphenol mono- and diethoxylate. 4-Nonylphenol was found in all samples and 4-nonylphenol monoethoxylate were found in every country.

The highest concentrations in sludge were observed for 4-nonylphenol and 4-nonylphenol mono- and diethoxylate.

4.4 Endosulfans

Endosulfan is an organochlorine compound that is used as an insecticide. Endosulfan consists of two isomers which differ in the configuration. These isomers are known as α -endosulfan and β -endosulfan. The ratio of α -endosulfan and β -endosulfan is approximately 2:1 in technical products. Endosulfan is acutely toxic, potential for bioaccumulation, and has role as an endocrine disruptor. Endosulfan is banned in several countries, including the European Union. Because of its threats to the environment, a global ban on the use and manufacture of endosulfan is being considered under the Stockholm Convention. Endosulfan breaks down into endosulfan sulphate and endosulfan diol. Endosulfan is subject to long range atmospheric transport.

Poland (IETU) analysed endosulfans for Germany, Latvia, Finland and Poland. Samples from Denmark were analysed by Eurofins. Estonian, Lithuanian and Swedish partners analysed their own samples, EERC, EPA and IVL, respectively. In this study α -endosulfan, β -endosulfan and endosulfan sulphate were analysed.

Analytical methods for effluents, storm waters and landfill leachates

The analytical methods for effluents, storm waters and landfill leachates varied between laboratories.

In Poland (IETU) an aliquot (1 L) of unfiltered effluent was passed through C-18 cartridge. After that, all cartridges were dried using vacuum. Endosulfans were eluted using ethyl acetate. The solvent excess was evaporated under the nitrogen stream and the final volume was set at 0.5 mL with ethyl acetate. Endosulfans were determined chromatographically using Shimadzu GCMS-QP2010S gas chromatograph with MS detector, column - Phenomenex ZB-5MS, 30m x 0.25mm ID, 0.25 μ m film thickness.

In Denmark (Eurofins) water samples were separated into liquid and solid phase by filtration. The internal standards (^{13}C -labeled β -endosulfan, endosulfan sulphate) were added afterwards. The liquid-liquid extraction with hexane/toluene was performed three times. The solid phase was hot extracted 8h with the solvent of the liquid-liquid extraction. The extracts were purified with florisil and basic alumina column. Thereafter the recovery standard (^{13}C -labeled PCB 105) was added. The extracts were concentrated (appr. 100 μ l) before instrumental analysis. A minimum of one blank was included. Endosulfans were determined gas chromatographically with high resolution mass spectrometry (HRGC/HRMS).

In Sweden (IVL) internal standards D4- α -endosulfan and D4- β - endosulfan were added and the sample was solid phase extracted (Oasis HLB). The analyte was eluted with MTBE/acetonitrile and hexane/MTBE. After solvent change to hexane the extract was applied on a silica gel (5 % water) column and eluted with hexane/MTBE. Endosulfans were determined on a GC 6890N interfaced to an MS 5973N (both Agilent) using chemical ionization mode with methane as reaction gas and selected ion monitoring.

In Estonia (EERC) liquid - liquid extraction with organic solvent (iso-octane + internal standard PCB189) was performed. Blanks, control samples and calibration solutions were analysed in all sequences. Endosulfans were determined by gas chromatograph equipped with ECD detector.

In Lithuania (EPA) 1 L of liquids was extracted by liquid-liquid extraction with hexane (recovery over 75%). Extraction was performed using a shaking machine and the extract was concentrated

using a rotary evaporator. The extract was cleaned with an alumina/silver nitrate column. The final result was corrected by recovery factor. The blank sample was determined in all sample series. Endosulfans were determined by gas chromatograph equipped with ECD detector.

Municipal waste water treatment plant

In total, 101 municipal effluent samples were collected for analysis of endosulfans. In the case of municipal effluent α -endosulfan was observed in 20% of samples. The maximum concentration (0.095 $\mu\text{g/l}$) was recorded in Latvia (Figure 4.21.).

β -endosulfan was found in 15% of municipal effluent samples. The maximum concentration (0.128 $\mu\text{g/l}$) was detected in Germany.

Endosulfan sulphate was observed in 24% of the municipal effluent samples. The maximum concentration (0.13 $\mu\text{g/l}$) was recorded in Poland.

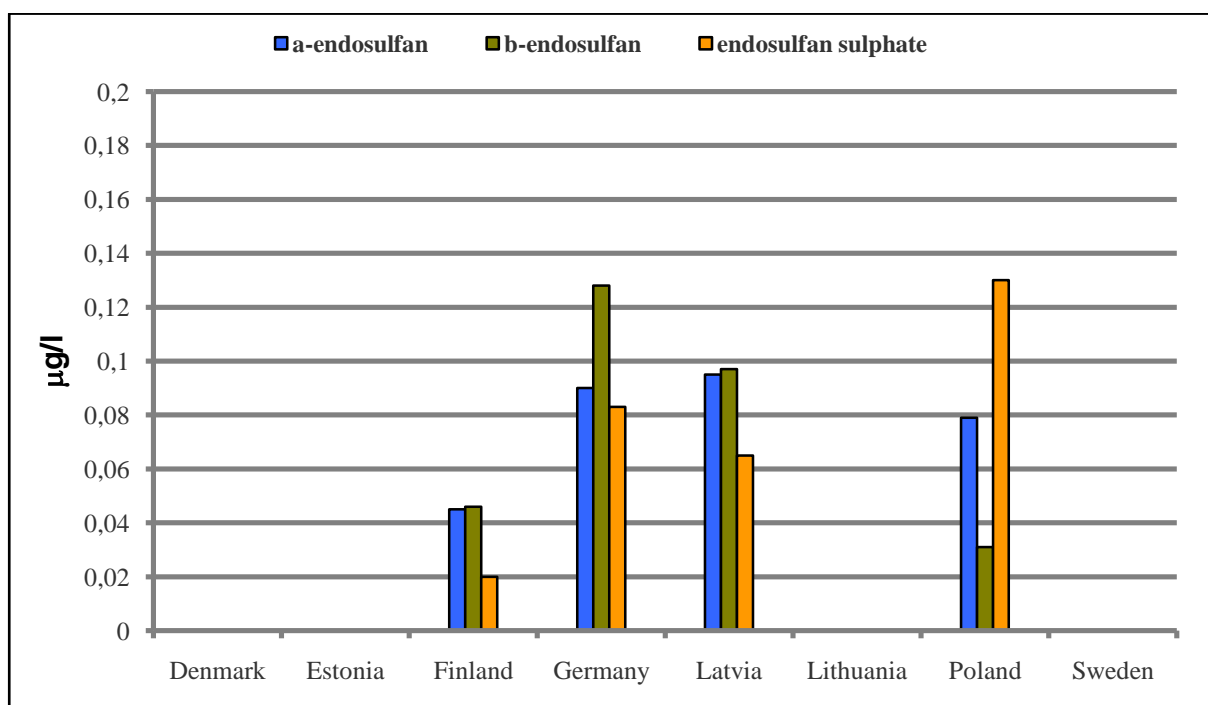


Figure 4.21. Maximum concentrations ($\mu\text{g/l}$) of endosulfans found in municipal effluents.

In municipal effluent samples the most frequently observed compound with the highest concentration was endosulfan sulphate. Endosulfans were not found in Denmark, Estonia, Lithuania and Sweden.

Industrial wastewater effluents

In total, 53 industrial effluent samples were collected for analysis of endosulfans. α -endosulfan was observed in 11% of the industrial effluent samples. The maximum concentration (0.11 $\mu\text{g/l}$) was found in Poland (Figure 4.22.). In 9% of the industrial effluent samples β -endosulfan were detected.

Similar to municipal effluents, the maximum concentration was recorded in Germany (0.20 µg/l). In the case of industrial effluent endosulfan sulphate was observed in 21% of the samples. The maximum concentration (0.045 g/l) was found in Germany.

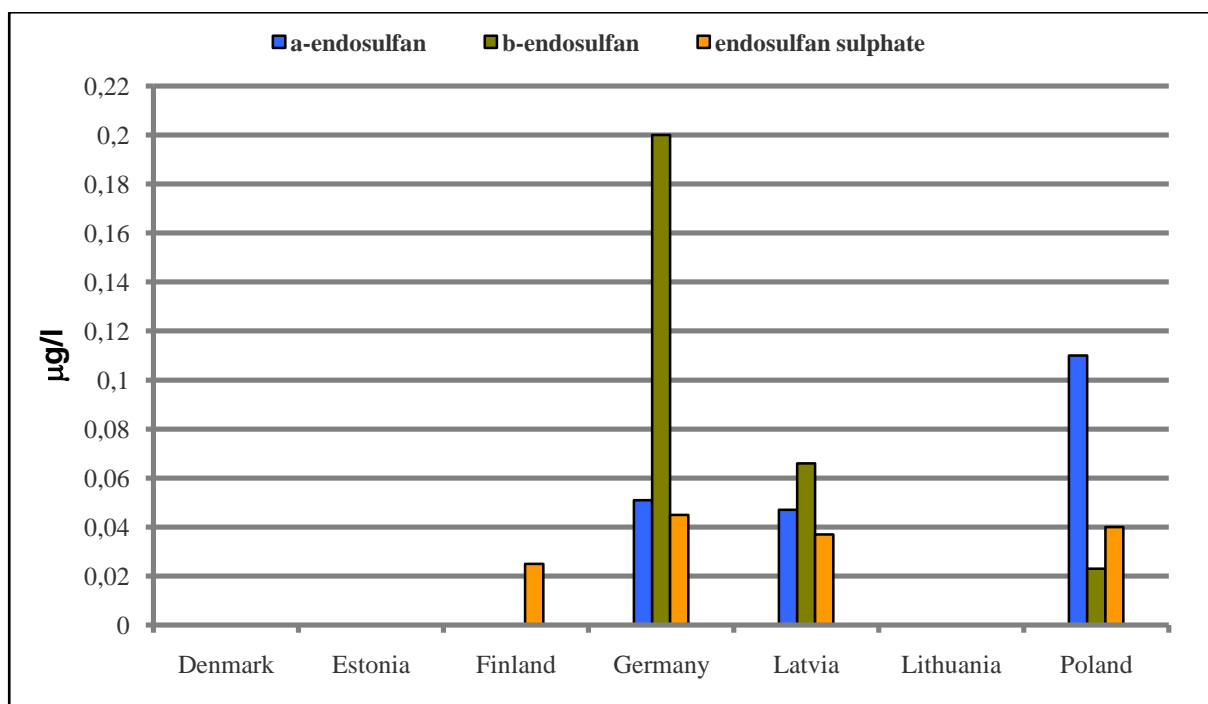


Figure 4.22. Maximum concentrations (µg/l) of endosulfans found in industrial effluents.

Endosulfan sulphate in industrial effluent samples was observed most frequently. Endosulfans were found in four countries (DE, FI, LV and PL). In industrial effluent samples the highest concentrations were recorded for endosulfan sulphate.

Storm water

Fourteen storm water samples were analysed for endosulfans and α -endosulfan was observed in 14% of the samples. The maximum concentration (0.028 µg/l) was found in Finland (Figure 4.23.). β -endosulfan was recorded in 7% of storm water samples and the maximum concentration (0.032 µg/l) was in Germany. In 14% of storm water samples the endosulfan sulphate was detected above LOQ, the maximum concentration (0.016 µg/l) was in Germany.

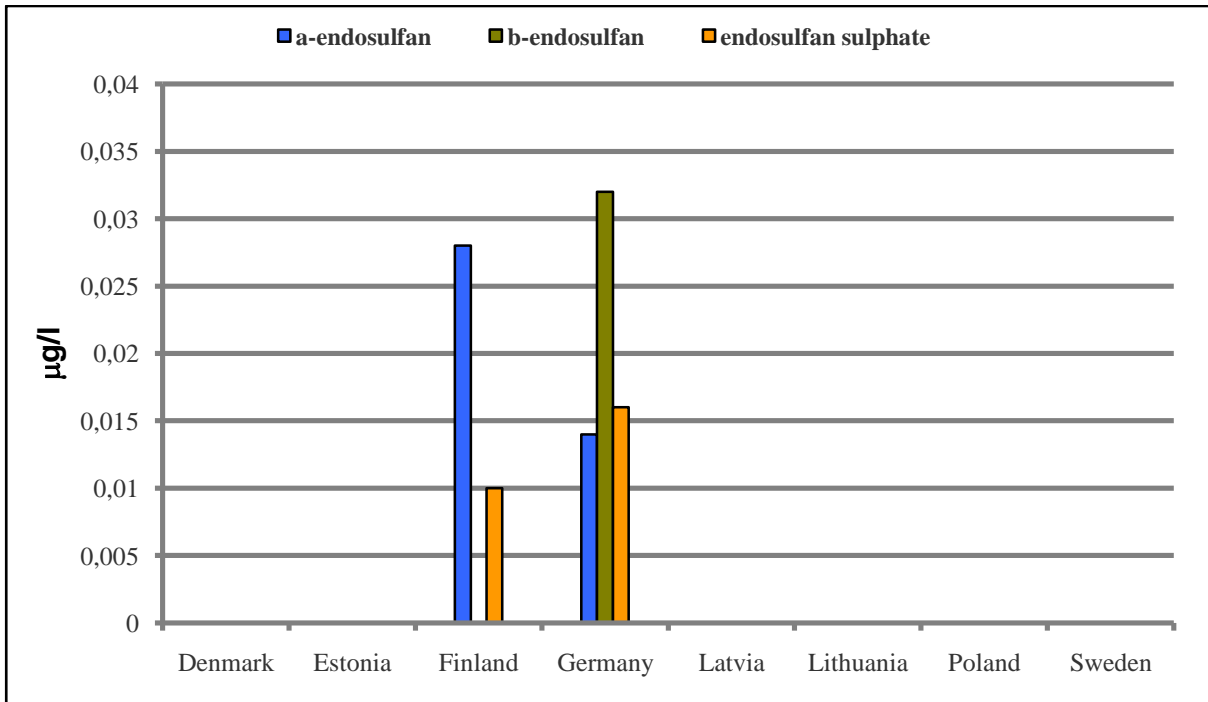


Figure 4.23. Maximum concentrations ($\mu\text{g/l}$) of endosulfans found in storm water.

In storm water samples α -endosulfan and endosulfan sulphate were equally frequently detected. Endosulfans were found only in Finland and Germany. In storm water samples the highest concentrations were recorded for β -endosulfan.

Landfill leachate

Fourteen samples were analysed for endosulfans. Concentrations of α - and β -endosulfan were not recorded (below the detection limit) in these samples. Endosulfan sulphate was observed in 29% of the samples, the maximum concentration ($0.16 \mu\text{g/l}$) was in Finland (Figure 4.24.). Finnish, Polish, Swedish, German and Danish leachates were untreated. In landfill leachate samples only endosulfan sulphate was observed in Estonia, Finland and Germany.

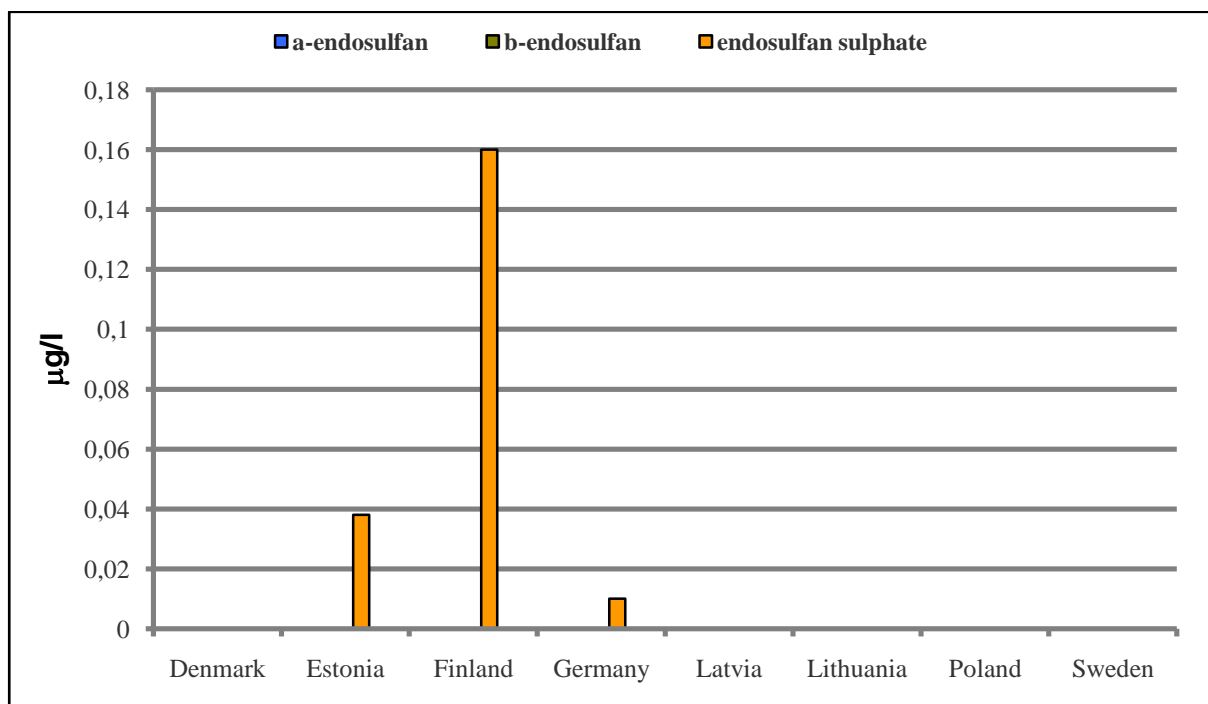


Figure 4.24. The maximum concentrations ($\mu\text{g/l}$) of endosulfans in landfill leachates.

Analytical methods for sludge

In Poland (IETU) the fresh sludge was air dried in a dark room and after grinding it was passed through a 0.25 mm sieve. An aliquot of air dried sludge was mixed thoroughly with diatomaceous earth, placed in an extraction cell. The obtained extract was evaporated under the nitrogen stream and then quantitatively transferred onto the top of the glass column filled with Florisil. Before use the column was rinsed with hexane/acetone solvent (1:1 v/v). Endosulfans were eluted using hexane/acetone solvent (1:1 v/v). The solvent excess was evaporated to approximately 0.3 ml under the nitrogen stream and the final volume was set at 0.5 ml with the same solvent. The parameters of chromatographic determination were the same as in the effluent analyses.

In Denmark (Eurofins) sludge samples were extracted with organic solvent with addition of the internal standard marked with ^{13}C . Extracts were cleaned chromatographically and analysed by gas chromatograph with high resolution mass spectrometry (HRGC/HRMS).

In Sweden (IVL) sludge was extracted with acetonitrile/hexane/MTBE. Water was added and the organic phase was cleaned chromatographically on silica gel and analysed as water samples.

In Estonia (EERC) the sludge sample was left to dry for 3 days in room temperature. The dry sample was homogenized using mortar and pestle. Then 3g of sample was placed into glass vessel and 1ml of internal standard stock solution PCB189-2 and 15 ml of n-hexane was added. Ultrasound assisted extraction (30 min) was repeated for three times. Then sample extract was concentrated using rotary evaporation and after a couple of minutes, 5 ml of sulphuric acid was added to remove any other organic compounds. Adding 5ml of n-hexane the acid was removed and the extract was again concentrated using rotary evaporation followed by addition of copper powder to remove any traces of sulphur for 30 min. After clean-up on silica gel column the sample extract was concen-

trated by rotary evaporation to dryness and 1ml of isooctane was added. Then sample extract was closed into glass vial and analysed by gas chromatography equipped with ECD detector.

In EPA (Lithuania) sludge samples were dried, grinded, homogenized and sieved if necessary. After that 10 g of the sample was weighed and extracted with petroleum ether/acetone (2:1) mixture. The extraction was performed using Soxhlet system. The extract was cleaned with an alumina column. Elemental sulphur was removed from the concentrated extract by the treatment with tetrabutylammonium sulphite solution.

Sludge

The quantification limits (LOQ) of endosulfans varied between laboratories (Table 4.20.). In some countries (DE, FI and PL) all observations were recorded (above the detection limit).

Table 4.20. LOQ (mg/kg d.w.) for endosulfans in sludge. In case where all observations were recorded the detection limit (mg/kg d.w.) is informed and limit of quantifications are in brackets.

	Denmark	Estonia	Sweden	Lithuania	Germany	Finland	Poland
α -endosulfan	0.001 (0.005)	(0.001)	(0.001)	0.0003 (0.001)		(0.01)	
β -endosulfan	0.001 (0.005)	(0.001)	(0.001)	0.0003 (0.001)		(0.01)	
endosulfan sulphate	0.001 (0.005)	(0.001)	(0.001)	0.0003 (0.001)		(0.01)	

Fifteen sludge samples analysed for endosulfans (Table 4.21.). In 53% of the samples α -endosulfan and β -endosulfan were detected. The maximum concentration of both compounds was found in Finland, 0.86 and 0.64 mg/kg d.w., respectively (Figure 4.25.). Endosulfan sulphate was recorded in 33% of the samples and the maximum concentration (1.45 mg/kg d.w.) was observed in Germany.

Table 4.21. Number of samples and the frequencies of endosulfan concentrations above LOQ in sludge.

	Number of samples	α -endosulfan	β -endosulfan	endosulfan sulphate
Denmark	2	0	0	0
Estonia	3	0	1	0
Finland	2	2	2	1
Germany	3	3	3	3
Latvia	0	0	0	0
Lithuania	1	1	0	0
Poland	2	2	2	1
Sweden	2	0	0	0
TOTAL	15	8	8	5
%		53	53	33

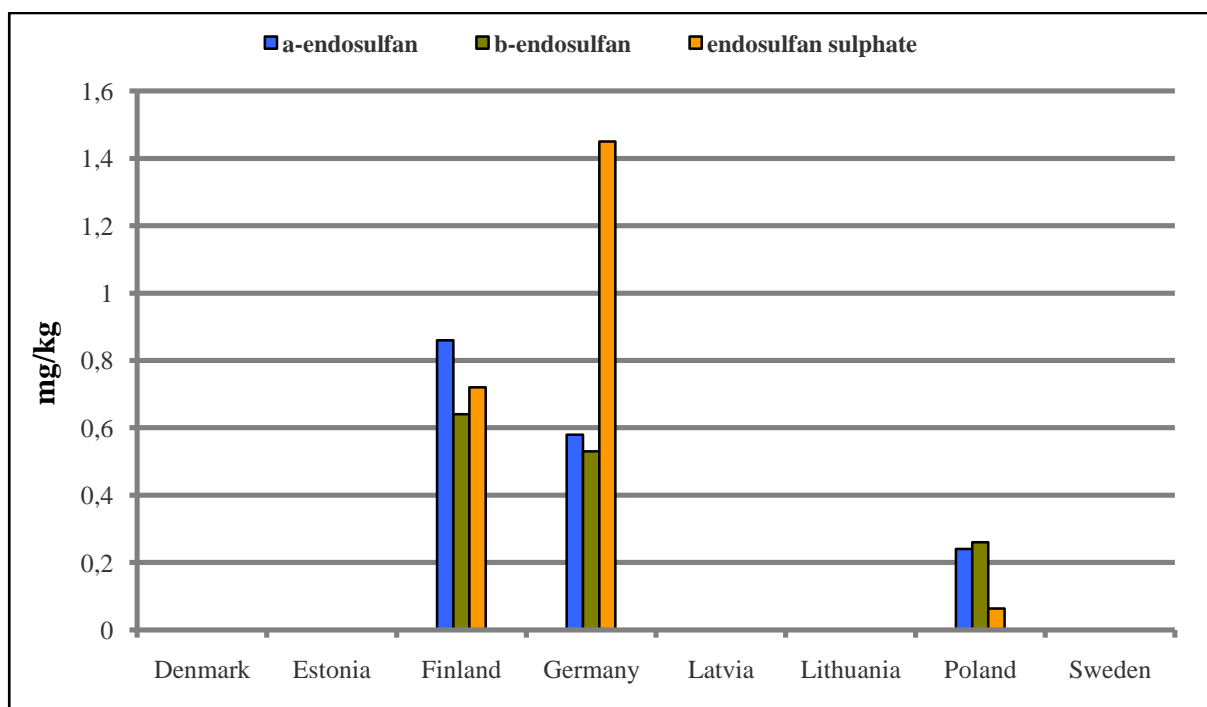


Figure 4.25. The maximum endosulfan concentrations (mg/kg d.w.) in sludge.

In Denmark, Latvia, Lithuania and Sweden endosulfans were not found in sludge at all. Beta-endosulfan was found from one sludge sample in Estonia and α -endosulfan in Lithuania. In the rest of the countries the frequency of detected endosulfans and endosulfan sulphate was equal. The highest endosulfan sulphate concentration in sludge was observed in Germany.

4.5 Dioxins, furans and PCBs

Dioxins (PCDDs, polychlorinated dibenzodioxins) are a group of polyhalogenated compounds comprising 75 different congeners. Dioxins have been shown to bioaccumulate in humans and wildlife due to their lipophilic properties, and are known to be teratogens, mutagens, and suspected human carcinogens. Dioxins occur as by-products in the manufacture of organochlorides, in the incineration of chlorine-containing substances such as PVC (polyvinyl chloride), in the bleaching of paper, and from natural sources such as volcanoes and forest fires.

Furans (PCDFs, polychlorinated dibenzofurans) are also toxic chemicals with properties and chemical structures similar to dioxins. Furans occur at low levels in commercial coal tars and as a by-product of smoking.

Dioxins are generally found in mixtures containing several kinds of dioxins and dioxin-like compounds and each of them has its own degree of toxicity. The International Toxic Equivalents (TEQ) scheme has been developed to express the overall toxicity of such mixtures. It has to be noticed that the TEQ scheme refers only to adverse effects by one mechanism, namely binding to the nuclear Ah receptor. The main focus has been the protection of humans. Other toxic effects of dioxins and dioxin-like compounds are not quantified by this scheme. In this report WHO-TEQ 2005 are used.

Analytical method for effluents, storm waters and landfill leachates

The analysing laboratory (THL, Finland) used liquid-liquid extraction and three columns (silica gel, aluminium oxide and carbon) for purification and separation. The final quantification was performed by gas chromatography - high resolution mass spectrometry (GC-HRMS).

Municipal effluents

Since the limit of quantification varied even between samples the lower bound (calculated as WHO-TEQ 2005) was used as limit for frequency studies. The observed lower bounds (WHO-TEQ, 2005) for dioxins, furans and co-PCBs in municipal effluents are in table 4.22.

Table 4.22. Observed lower bounds (WHO-TEQ 2005) for dioxins, furans and co-PCBs in municipal effluents (n.i. not informed).

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	0.72 - 92	n.i.	0 - 0.0551	n.i.	0 - 0.1396	0.0001 - 0.14	0 - 0.069	n.i.
co-PCB	4.8 - 188	n.i.	0 - 0.0362	n.i.	0.9481 - 9.3989	0.0001 - 3.3647	0.00003 - 0.02	n.i.

Altogether 40 municipal effluents were analysed for dioxins, furans and co-PCBs (Table 4.23.). Dioxins and furans were found in 72% of the samples. The maximum concentration (3.1 pg/l) was found in Germany (Figure 4.26.). The maximum co-PCB concentration (0.66 pg/l) was found in Germany, and co-PCBs were detected in 72% of the samples.

Table 4.23. Number of samples and the calculated frequencies of dioxin, furan and co-PCB findings in municipal effluents (n.i. not informed).

	Number of samples	PCDD/F	co-PCB
Denmark	6	4	2
Estonia	6	4	6
Finland	9	7	7
Germany	4	2	2
Latvia	6	6	6
Lithuania	6	1	n.i.
Poland	9	9	9
Sweden	4	4	4
TOTAL	50	37	36
%		74	72

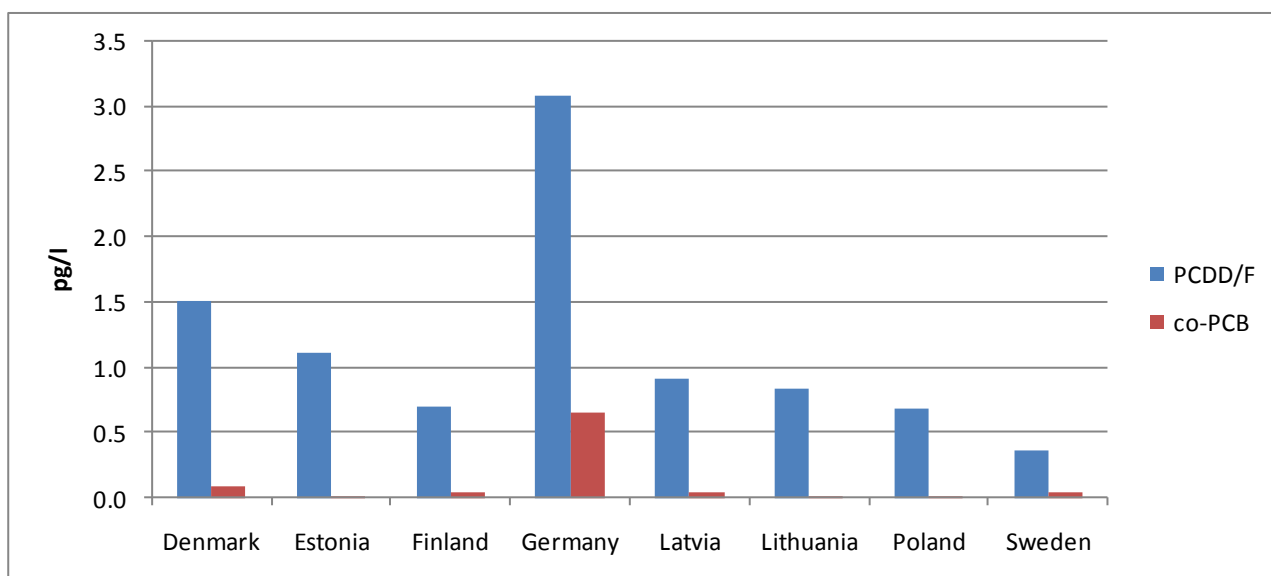


Figure 4.26. The maximum dioxin, furan and co-PCB (pg/l) concentrations found in municipal effluents. Maximum values are expressed as WHO-TEQ 2005 upperbound.

Industrial effluents

Similar to municipal effluent samples the lower bound (calculated as WHO-TEQ 2005) was used for frequency studies. The observed lower bounds for dioxins, furans and co-PCBs in industrial effluents are in table 4.24.

Table 4.24. The lower bounds (WHO-TEQ 2005) for dioxins, furans and co-PCBs in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	0.72 - 520		0.00185 - 0.03853	0.0044 - 0.3546	0 - 0.9804	0.0498 - 0.4862	0.72 - 520	-
co-PCB	4.8 - 190		0 - 0.0193		0 - 0.1605	0.0003 - 0.0170	0 - 0.1	-

From all the 31 industrial effluents, dioxins and furans were quantified in 65% of the samples, while co-PCBs were found in 61% (Table 4.25.). The maximum concentration of dioxin/furans and co-PCBs was found in Germany (6.7 and 1.1 pg/l) (Figure 4.27.).

Table 4.25. The number of samples and frequencies of dioxin, furan and co-PCB findings of industrial effluents (n.i. not informed).

	Number of samples	PCDD/F	co-PCB
Denmark	4	3	2
Estonia	6	4	6
Finland	3	3	2
Germany	3	1	1
Latvia	6	6	5
Lithuania	6	n.i.	n.i.
Poland	3	3	3
Sweden	0	-	-
TOTAL	31	20	19
%		65	61

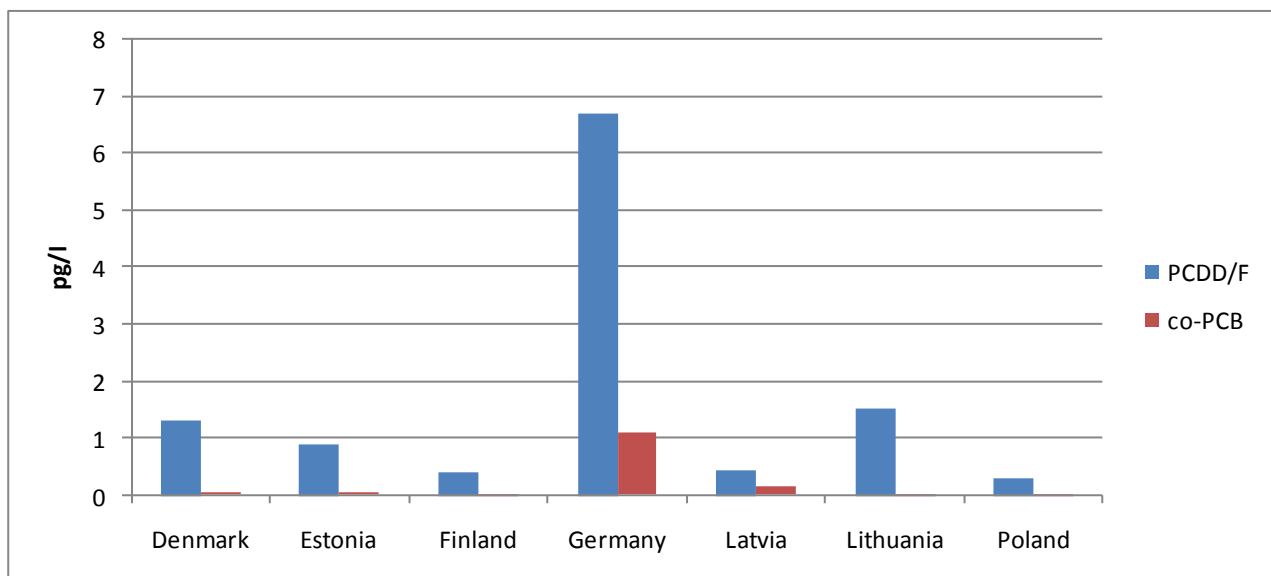


Figure 4.27. The WHO-TEQ 2005 upperbound values (pg/l) found in industrial effluents.

Storm water

Similar to municipal and industrial effluent samples the lower bound (calculated as WHO-TEQ 2005) was used for frequency studies. The observed lower bounds for dioxins, furans and co-PCBs in storm waters are shown in table 4.26.

Table 4.26. The calculated lower bounds (WHO-TEQ 2005) for dioxins, furans and co-PCBs in storm waters (n.i. not informed).

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	0.72-1.92	n.i.	0.1492	n.i.	2.3197	0	0.099	n.i.
co-PCB	4.8-24	n.i.	0.0381	n.i.	0.8849	0.0009	0.0004	n.i.

Nine storm water samples were collected for analysis of dioxins, furans and co-PCBs (Table 4.27.). Dioxins and furans were found in 8 of the 9 storm water samples. The maximum concentration (11 pg/l) was found in Germany (Figure 4.28.). co-PCBs were found in 89% of taken storm water samples. The maximum concentration (18 pg/l) was found in Germany.

Table 4.27. Number of samples and noted frequencies of dioxins, furans and co-PCBs in storm water (n.i. not informed).

	Number of samples	PCDD/F	co-PCB
Denmark	2	2	2
Estonia	1	1	1
Finland	1	1	1
Germany	1	1	1
Latvia	1	1	1
Lithuania	1	0	n.i.
Poland	1	1	1
Sweden	1	1	1
TOTAL	9	8	8
%		89	89

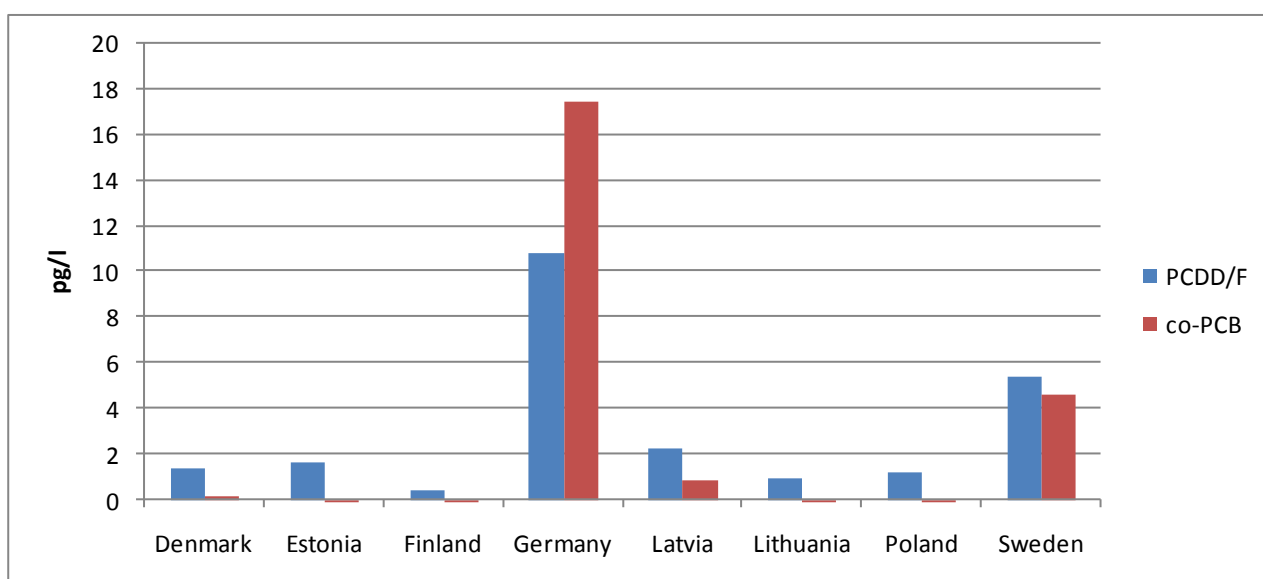


Figure 4.28. The WHO-TEQ 2005 upperbound values (pg/l) found in storm waters.

Landfill leachate

Like in other analysed samples the calculated WHO-TEQ 2005 lower bound for dioxins, furans and co-PCBs in landfill leachate are presented in table 4.28. Dioxins/furans and co-PCBs were found in 75% and 88% of the 8 landfill leachate samples. The highest WHO-TEQ values (13 and 2.3 pg/l) were found in Poland. (Figure 4.29.). Leachates were untreated in all other countries except Estonia, Lithuania and Latvia. Results are based on one sampling only, except Denmark with two samplings (Table 4.29.).

Table 4.28. The calculated lower bounds (WHO-TEQ 2005) for dioxins, furans and co-PCBs in landfill leachate (n.i. not informed).

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	0.72 - 30	n.i.	4.6576	-	0.1288	0	13.23	n.i.
co-PCB	5.0 - 60	n.i.	0.9614	-	0.1583	0.0001	2.268	n.i.

Table 4.29. Number of samples and found frequencies of dioxins, furans and co-PCBs in landfill leachate (n.i. not informed).

	No of samples	PCDD/F	co-PCB
Denmark	2	1	2
Estonia	1	1	1
Finland	1	1	1
Germany	0	-	-
Latvia	1	1	1
Lithuania	1	n.i.	n.i.
Poland	1	1	1
Sweden	1	1	1
TOTAL	8	6	7
%		75	88

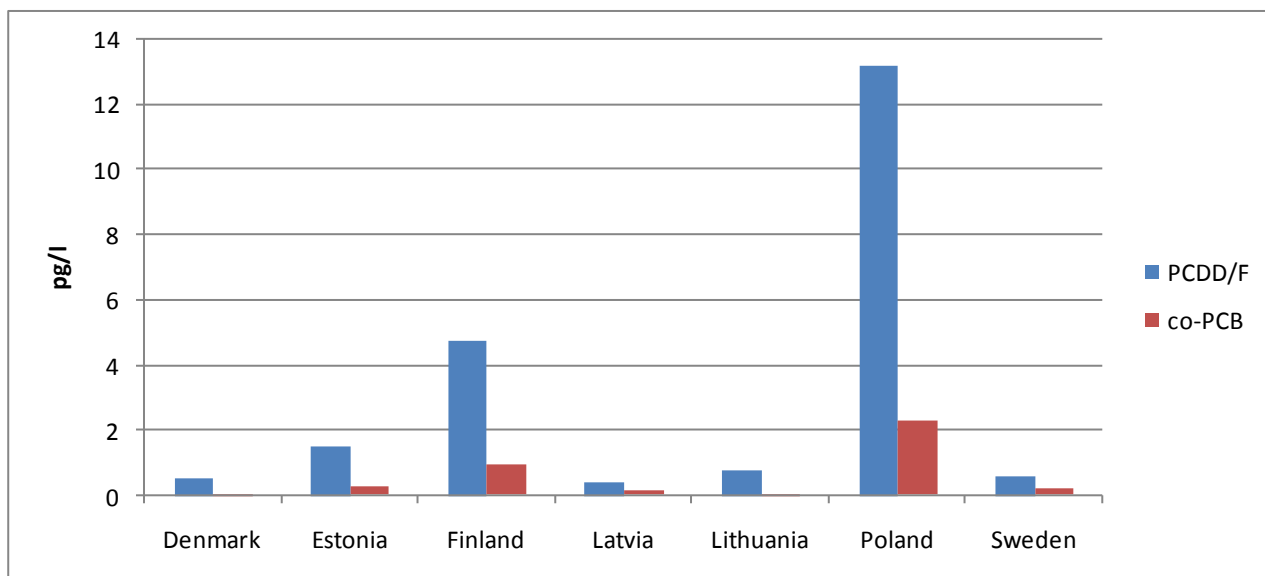


Figure 4.29. Maximum concentrations (pg/l) found in landfill leachate. Maximum values are expressed as WHO-TEQ 2005 upper bound.

Analytical method for sludge

THL laboratory used Soxhlet extraction and three columns (silica gel, aluminium oxide and carbon) for purification and separation. The final quantification was performed by gas chromatography - high resolution mass spectrometry (GC-HRMS).

In Denmark the determination of dioxins, furans and co-PCBs in sewage sludge were made according to the German Sewage Sludge Ordinance (AbfKlärV) by means of high resolution mass spectrometry (HRGC/HRMS).

Sludge

Thirteen sludge samples were analysed for dioxins, furans and co-PCBs (Table 4.30.). Since the limit of quantification varied even between samples the lower bound (calculated as WHO-TEQ 2005) was used as limit for frequency studies. The observed lower bounds (WHO-TEQ 2005) for dioxins, furans and co-PCBs in sludge are in table 4.31.

Table 4.31. Observed lower bounds (WHO-TEQ 2005) for dioxins, furans and co-PCBs in sludge (n.i. not informed).

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	0.27 - 0.80	-	3.739	n.i.	8.4349 - 14.7970	2.9889	10.69	n.i.
co-PCB	3.61 - 3.99	-	0.7934	n.i.	2.2653 - 2.3149	0.6507	1.186	n.i.

Dioxins/furans and co-PCBs were found in 69% of samples (Table 4.30.). The highest WHO-TEQ concentrations were found in Germany 770 ng/kg dioxins/furans and 79 ng/kg co-PCBs (Figure 4.30.).

Table 4.30. Number of samples and found frequencies of dioxins, furans and co-PCBs in sludge.

	Number of samples	PCDD/F	co-PCB
Denmark	4	4	4
Estonia	0	-	-
Finland	1	1	1
Germany	1	1	1
Latvia	2	2	2
Lithuania	1		
Poland	2	1	1
Sweden	2		
TOTAL	13	9	9
%		69	69

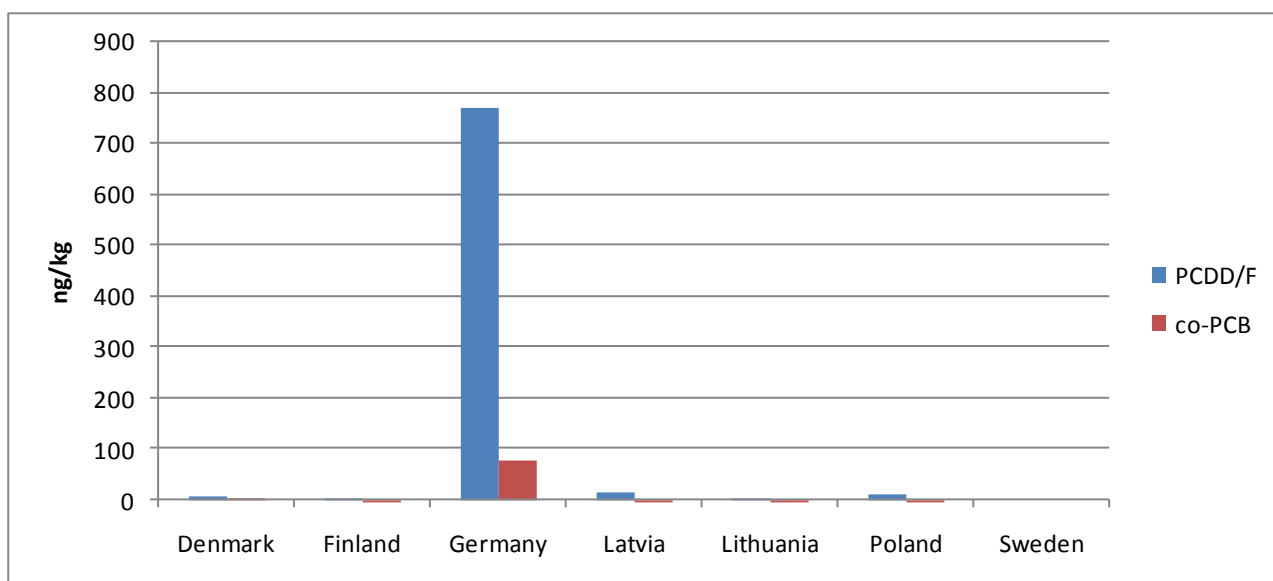


Figure 4.30. The maximum concentrations (ng/kg) expressed as WHO-TEQ 2005 upperbound of dioxins/furans and co-PCBs found in sludge.

4.6 Chlorinated paraffins

Chlorinated paraffins are complex mixtures of n-alkanes homologues with variable chain lengths and chlorine contents. There are several thousands of congeners, homologues, isomers and enantiomers. Properties of the molecules depend on the length of the carbon chain of the paraffin molecules used and on the proportion of chlorine added.

Short chain chlorinated paraffins SCCPs (C₁₀-C₁₃) and medium chain chlorinated paraffins MCCPs (C₁₄-C₁₇) are liquids. SCCPs are only allowed to be used in mining conveyor belts. Medium chain chlorinated paraffins are used as flame retardants in rubber, in flexible plastics, in certain textiles and in other specific applications.

Analytical methods for effluents, storm water and landfill leachate

The method described below was applied for samples collected in Estonia, Finland, Latvia, Germany, Lithuania, Sweden and Poland. Analyses of SCCPs were made by IETU lab, whereas analyses of MCCPs were determined by Institute of Non-Ferrous Metals (INM). Chlorinated paraffins were determined chromatographically with μ -ECD detector. In Denmark analysis of SCCP and MCCP were performed by Eurofins GfA GmbH in Germany.

For SCCP analyses an aliquot of unfiltered effluent was passed through C-18 cartridge. Before use the cartridges were washed consecutively with 6 ml of methanol and 6 ml of distilled water. The effluent sample was passed through the cartridge at a flow rate of about 5 ml/min. After that, all cartridges were air dried using vacuum for 30 min. SCCPs were eluted using 10 ml of hexane. The solvent in the eluate was evaporated to approximately 0.5 ml under the nitrogen stream. Due to a strong matrix all samples were cleaned-up in the next SPE procedure with cartridges filled with 1 g of Amino (NH₂) and 1 g of Cyan (CN) phases. Before use the cartridges were washed with 10 ml of hexane. The concentrated eluate from the previous cleaning stage was quantitatively transferred onto the top of the cartridge. SCCPs were eluted by passing 10 ml of hexane through the cartridge. The solvent excess was evaporated to approximately 0.3 ml under nitrogen stream and the final volume was set at 0.5 ml using hexane.

For MCCP analyses liquids (water matrix) were filtrated. C-18 cartridges were used. Before use the cartridges were washed with 2 ml of methanol and 2 ml of distilled water. One litre of water samples were passed through the cartridges at a flow rate of about 5ml/min. After that, cartridges were vacuum-dried for 15 min. Chlorinated paraffins were eluted using 6 ml hexane. The solvent in the eluate was evaporated just to dryness and reconstituted in 1 ml of hexane. Detailed descriptions of analytical methods can be found in the national reports and ring-test report (National COHIBA WP 3 report of Poland).

In Denmark chlorinated paraffins were analysed by Eurofins GfA GmbH in Germany. Water samples were extracted as whole samples. After addition of the internal standard (cis-chlordane) liquid-liquid extraction with toluene was performed three times. The extracts were purified with sulphuric acid and alumina column (2 % water). After addition of the recovery standard (trans-chlordane) extracts were concentrated (appr. 100 μ l) before instrumental analysis. Gas chromatography with low resolution mass spectrometry (HRGC/LRMS) was used for instrumental analysis of the chlorinated alkanes. A minimum of one blank was included (National COHIBA WP 3 report of Denmark).

Municipal effluent

Limit of quantifications (LOQ) varied between laboratories and chloroparaffins (Table 4.32.). In some countries observations below LOQ but above detection limit were also recorded.

Table 4.32. LOQ ($\mu\text{g/l}$) for chloroparaffins in municipal effluents. If all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and the limit of quantification is given in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	0.0002 (0.005)	0.02 (0.07)						
MCCP	0.0006 (0.008)	0.2 (0.6)						

In total 106 and 84 municipal effluent samples were analysed for SCCPs and MCCPs, respectively (Table 4.33.). SCCPs were observed in 92% of samples. The maximum concentration ($2.7\mu\text{g/l}$) was noted in Poland (Figure 4.31.). Medium chain chlorinated paraffins were recorded in 93% of municipal effluent samples. The maximum concentration ($31.5\mu\text{g/l}$) was found in Lithuania.

Table 4.33. Number of samples and noted frequencies of chloroparaffins observed in municipal effluents.

	Number of samples	SCCP	No of samples	MCCP
Denmark	6	0	6	4
Estonia	12	12	10	10
Finland	18	18	10	10
Germany	12	12	10	10
Latvia	4	3	3	3
Lithuania	12	12	20	9
Poland	18	18	15	14
Sweden	24	23	20	18
TOTAL	106	98	84	78
%		92		93

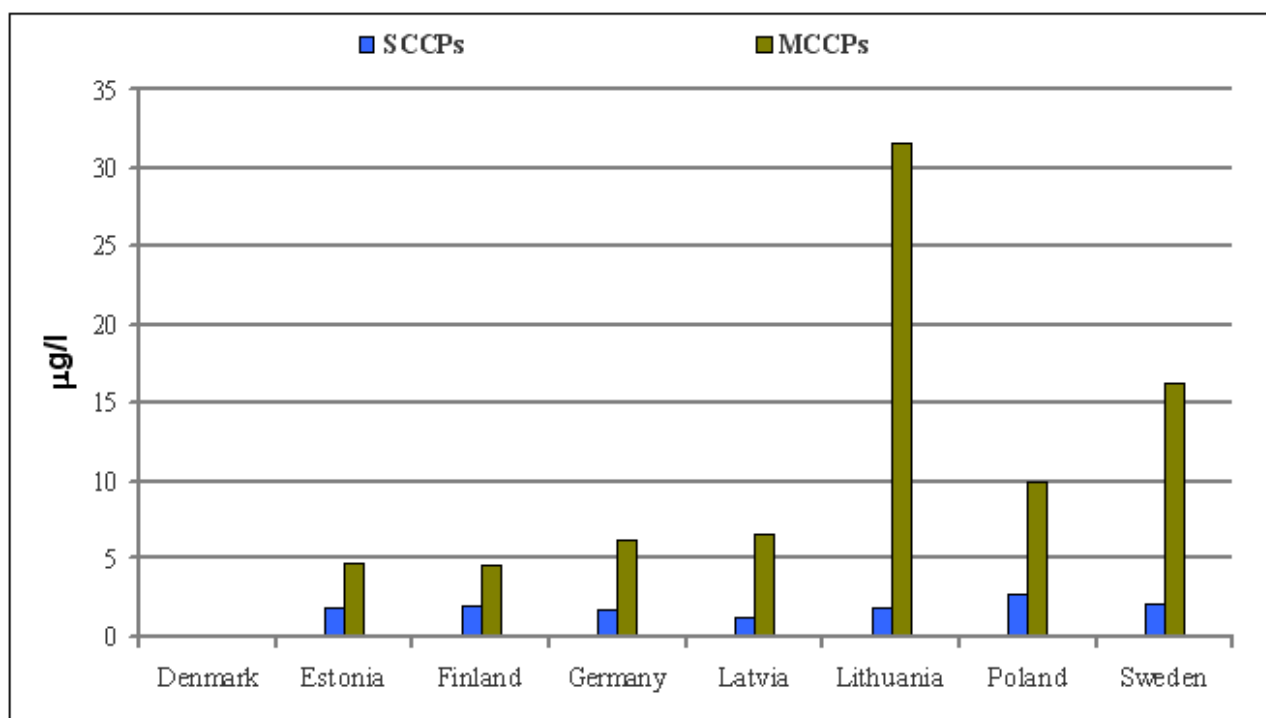


Figure 4.31. Maximum concentrations ($\mu\text{g/l}$) of chloroparaffins found in municipal effluents.

In municipal effluent samples SCCPs and MCCPs were equally frequently found, they were found in every country. In Denmark the measured maximum concentration of MCCP was $0.05 \mu\text{g/l}$ in municipal effluent.

Industrial effluents

In all countries except Denmark, all SCCP observations, also below LOQ were recorded (above the detection limit, Table 4.34.).

Table 4.34. LOQ ($\mu\text{g/l}$) for chloroparaffins in industrial effluent. If all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and the limit of quantification is given in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	0.0008 (0.006)					0.02 (0.07)		
MCCP	0.0012 (0.0062)					0.2 (0.6)		

In total, 53 industrial effluent samples were analysed for SCCPs and 46 for MCCPs (Table 4.35.). SCCPs were observed in 91% of the samples, the maximum concentration ($3.6 \mu\text{g/l}$) was found in Latvian effluent (Figure 4.32.). MCCPs were recorded in 98% of the samples. The maximum concentration ($15.9 \mu\text{g/l}$) was detected in Poland.

Table 4.35. Frequencies of chloroparaffin concentrations above LOQ in industrial effluents.

	Number of samples	SCCP	Number of samples	MCCP
Denmark	4	1	4	3
Estonia	12	12	10	10
Finland	6	6	5	5
Germany	11	10	10	10
Latvia	2	1	2	2
Lithuania	12	12	10	10
Poland	6	6	5	5
Sweden	0	-	0	-
TOTAL	53	48	46	45
%		91		98

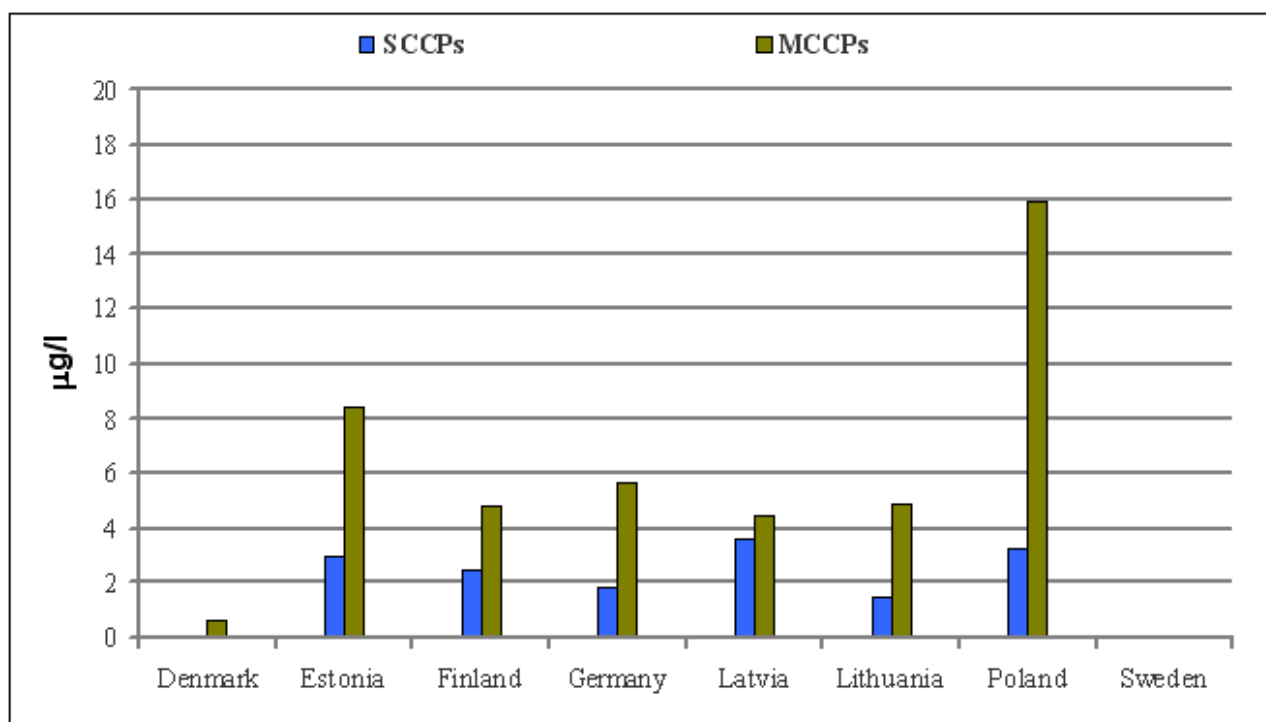


Figure 4.32. Maximum concentrations ($\mu\text{g/l}$) of chloroparaffins found in industrial effluents.

Storm water

Chlorinated paraffins were analysed from 15 storm water samples (Table 4.36.). Limit of quantifications (LOQ) are shown in table 4.37. SCCPs were observed in 93% of the samples and the maximum concentration ($4.8\mu\text{g/l}$) was recorded in Sweden (Figure 4.33.). MCCPs were found in 80% of the storm water samples and the maximum concentration ($3.6\mu\text{g/l}$) was found in Lithuania.

Table 4.37. LOQ ($\mu\text{g/l}$) for chloroparaffins in storm water. In the case all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and the limit of quantification is given in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	0.0006 (0.0073)					0.02 (0.07)		
MCCP	0.0013 (0.0102)					0.2 (0.6)		

Table 4.36. Number of samples and frequencies of findings of chlorinated paraffin in the storm water samples.

	Number of samples	SCCP	MCCP
Denmark	2	1	1
Estonia	2	2	2
Finland	2	2	1
Germany	2	2	2
Latvia	1	1	1
Lithuania	2	2	2
Poland	2	2	2
Sweden	2	2	1
TOTAL	15	14	12
%		93	80

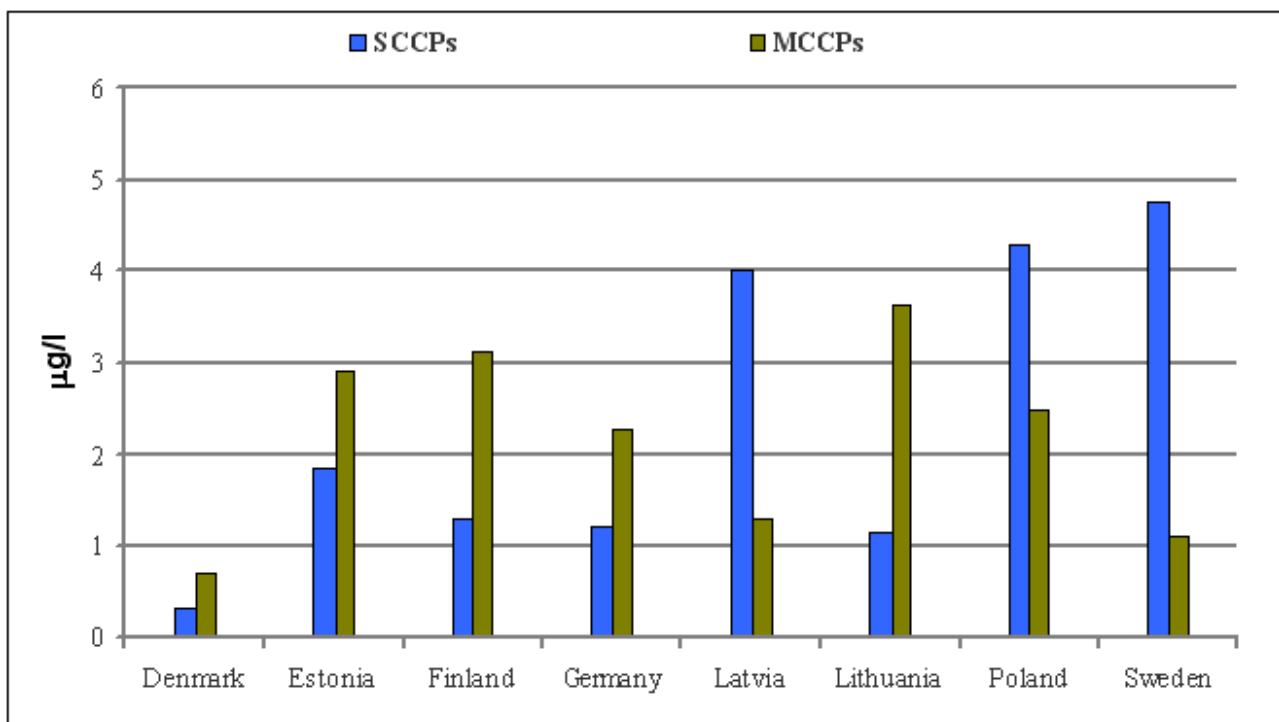


Figure 4.33. Maximum concentrations ($\mu\text{g/l}$) of chlorinated paraffins found in storm water.

Landfill leachate

Chlorinated paraffins were analysed from 15 landfill leachate samples (Table 4.38.). LOQs are shown in table 4.39. SCCPs were observed in 93% of the samples and the maximum concentration (10.9 µg/l) was recorded in Poland (Figure 4.34.). MCCPs were found in 62% of the samples and the maximum concentration 21.0µg/l) was found in Poland.

Table 4.39. LOQ (µg/l) for chlorinated paraffins in landfill leachate. If all observations were recorded, the detection limit (µg/l) is presented, the limit of quantification is in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	0.003 (0.008)	0.02 (0.07)						
MCCP	0.0025 (0.014)	0.2 (0.6)						

Table 4.38. Number of samples and frequencies of chlorinated paraffin concentrations above LOQ in landfill leachate.

	Number of samples	SCCP	Number of samples	MCCP
Denmark	2	1	2	1
Estonia	2	2	1	0
Finland	2	2	1	0
Germany	2	2	2	2
Latvia	1	1	1	0
Lithuania	2	2	2	2
Poland	2	2	2	2
Sweden	2	2	2	1
TOTAL	15	14	13	8
%		93		62

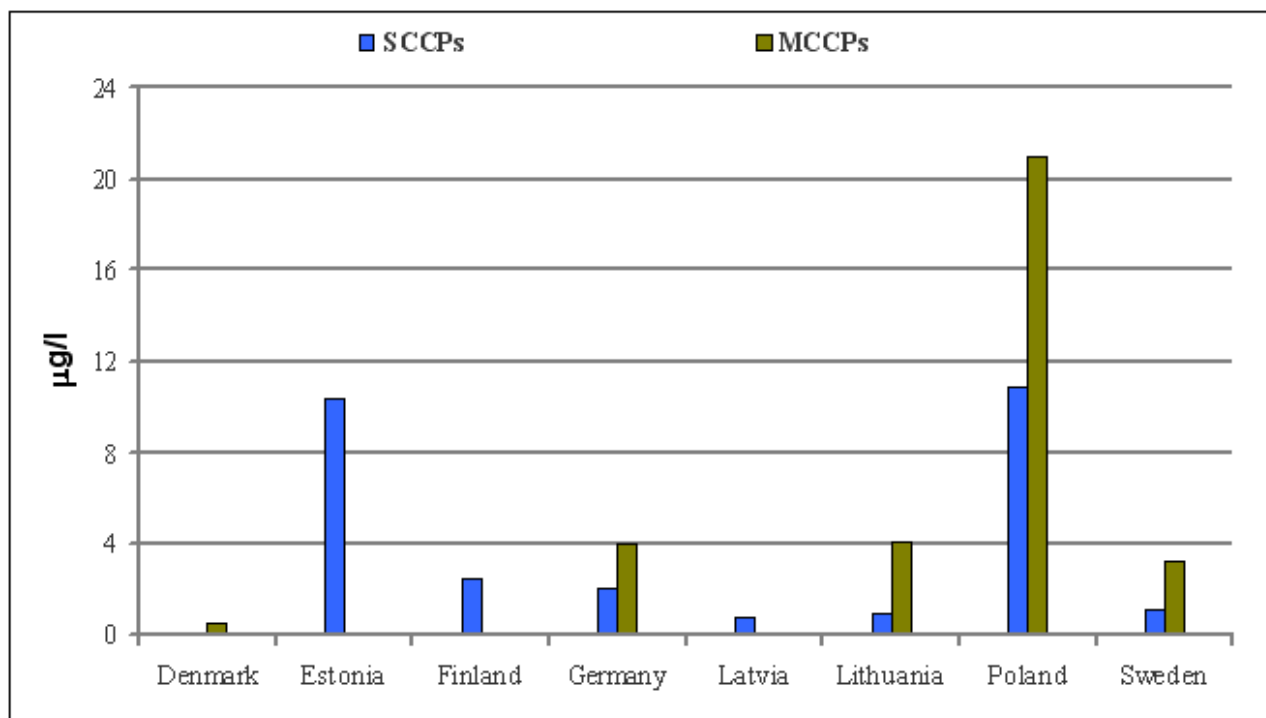


Figure 4.34. The maximum concentrations ($\mu\text{g/l}$) of chlorinated paraffins found in landfill leachates.

Analytical methods for sludge

Chlorinated paraffins SCCPs ($\text{C}_{10}\text{-C}_{13}$) and MCCPs ($\text{C}_{14}\text{-C}_{17}$) were determined chromatographically equipped with $\mu\text{-ECD}$ detector.

Pre-treatment of samples for determination of SCCPs in sludge was carried out in the following way: the fresh sludge was air dried in a dark room and after grinding it was passed through a 0.25 mm sieve. An aliquot of air dried sludge (2 g) was mixed thoroughly with 2 g of diatomaceous earth, placed in an extraction cell and mounted in the tray extractor (Accelerated Solvent Extractor). Subsequent pre-treatment steps were conducted in the same way as in the case of effluent samples.

Sludge samples were extracted for MCCP analyses in the following way: 10 g sample was extracted in the ultrasonic bath in 75 ml of dichloromethane for 1 h. The extract was filtrated and evaporated nearly to dryness on the vacuum evaporator. Before the chromatographic analysis, the extract was reconstituted with 1 ml of hexane and cleaned from sulphur by adding copper. Detailed descriptions of analytical methods can be found in the national reports and ring-test report (National COHIBA WP 3 report of Poland).

Danish sludge samples for SCCPs and MCCPs were analysed by Eurofins GfA GmbH in Germany. Samples were extracted with toluene with addition of an internal standard (cis-Chlordane and trans-Chlordane) as a recovery standard. Extracts were cleaned up by column chromatography and analysed by gas chromatography coupled with mass spectrometry (GC/MS-NCI).

Sludge

Sixteen sludge samples were analysed of chloroparaffins (Table 4.40.). SCCPs were observed in all sludge samples. The highest concentration (14.1 mg/kg d.w.) was found in Sweden (Figure 4.35.). MCCPs were recorded in 50% of the samples analysed. The highest concentration (37.6 mg/kg d.w.) was found in Denmark.

LOQs of chlorinated paraffins are listed in table 4.41. In Denmark, Estonia, Finland, Lithuania and Sweden all observations were recorded (above the detection limit).

Table 4.41. Limit of quantifications (mg/kg d.w.) for chloroparaffins in sludge. If all observations were recorded, the detection limit (mg/kg d.w.) is presented, the limit of quantification is in parenthesis.

	Denmark	Estonia	Finland	Germany	Lithuania	Poland	Sweden
SCCP	0.0007 (0.005)	0.06 (0.20)					
MCCP	Not informed	0.01 (0.03)					

Table 4.40. Number of samples and recorded frequencies of chloroparaffin concentrations above LOQ in sludge.

	Number of samples	SCCP	MCCP
Denmark	4	4	4
Estonia	3	3	3
Finland	2	2	1
Germany	3	3	0
Latvia	0	-	-
Lithuania	2	2	1
Poland	2	2	0
Sweden	2	2	1
TOTAL	18	18	10
%		100	50

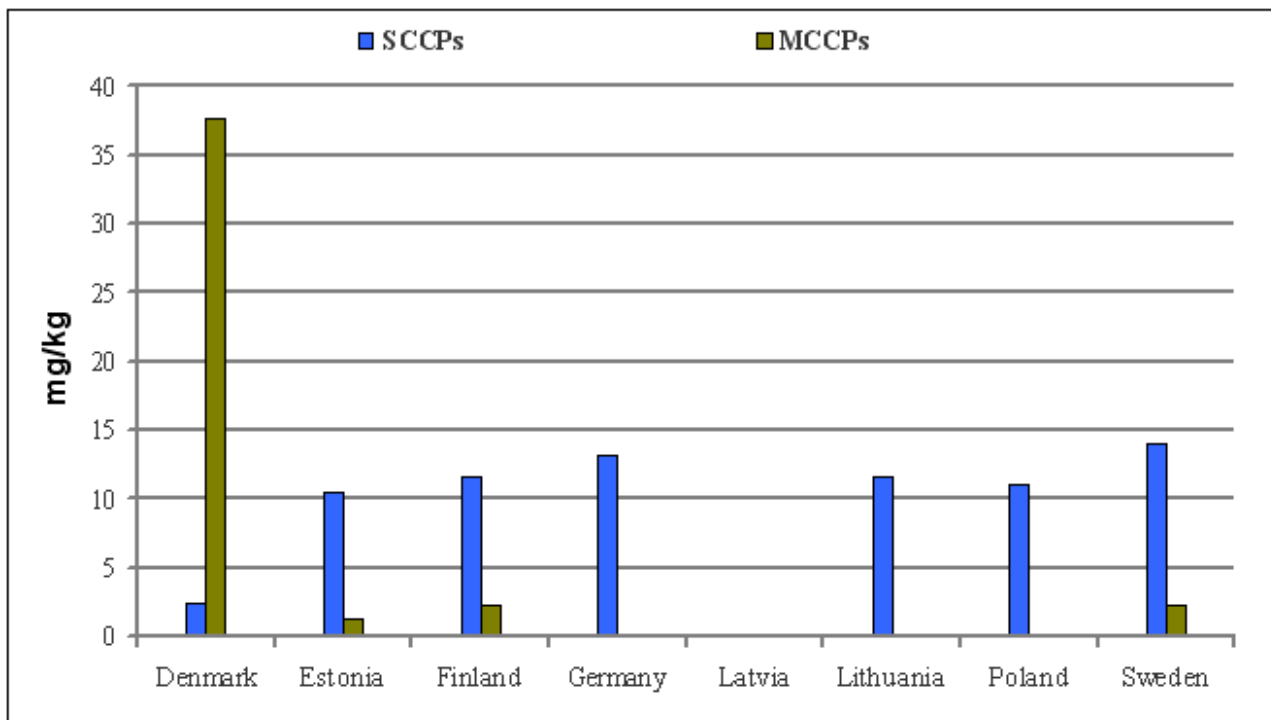


Figure 4.35. Maximum concentrations (mg/kg d.w.) of chloroparaffins found in sludge.

4.7 Brominated flame retardants

4.7.1 Polybrominated diphenylethers, PBDEs

Polybrominated diphenylethers are a group of substances with varying degrees of bromination. There are altogether 209 possible congeners with varying chemical properties and biological activities. PBDEs are used as flame retardants in a wide variety of products. The use of penta- and octaBDE is banned in EU but inflow to EU market is occurring via importing *e.g.* electronic circuits, textiles and plastics.

Pentabromodiphenyl ether (pentaBDE), octabromodiphenyl ether (octaBDE), and decabromodiphenyl ether (decaBDE) are mentioned in BSAP as substances of specific concern to the Baltic Sea. Commercially available PBDEs are mixtures of congeners. Finland (SYKE) analysed polybrominated diphenylethers for Finland, Estonia, Germany, Latvia, Lithuania and Poland. Sweden (IVL) and Denmark (Eurofins) analysed their own samples. In this study pentaBDE (congeners are 28, 47, 99, 100, 153, and 154), octaBDE (congeners 183 and 203) and decaBDE (congener 209) were analysed. In addition congeners 17, 66 and 85 were analysed.

Analytical method for effluents, storm water and landfill leachate

The analytical methods for effluents, storm water and landfill leachate varied between laboratories. In SYKE water samples (approx. 3200 ml) were extracted as whole samples. The liquid-liquid extraction with dichloromethane was performed two times to attain good recoveries (over 70%). The extracts were cleaned with multilayer silica column and basic alumina column (according ISO 22032). The samples were concentrated and solvent was changed to nonane before instrumental

analysis. Low pressure gas chromatography tandem mass spectrometry (LP-GC-(TQ)MS/MS) was used for instrumental analysis of PBDE compounds. Isotopic dilution technique using ¹³C-labelled PBDE compounds as internal standards were applied for quantification and recovery calculations. The final results are recovery corrected. A blank sample was analysed in each sample batch.

In IVL water samples (1000 ml) were filtered (glass fibre filter). The filtrate was solid phase extracted (C18 column). The column was eluted with acetone to which water was added. The analytes were extracted with hexane. The filter was extracted with acetone and hexane:MTBE. Extracts were combined and treated with sulphuric acid and fractionated on silica gel. Internal standards were BDE-119, 166, 190. GC/ECNI-MS with methane as reaction gas was used to monitor m/z 79 and 81.

In Eurofins water samples were extracted as whole samples (800 ml). The internal standards (eight ¹³C-labelled PBDE compounds) were added before liquid-liquid extraction with toluene (three times). The extracts were purified with sulphuric acid and basic alumina column (according ISO 22032). The extracts were concentrated (50 µl) and recovery standard (¹³C-labelled PBDE compound) was added before instrumental analysis. Gas chromatography with low resolution mass spectrometry (HRGC/LRMS) was used for instrumental analysis of PBDE compounds. The final results are recovery corrected. A minimum of one blank is included.

Municipal effluents

LOQ varied between laboratories and congeners (Table 4.42.). If LOQs varied between the congeners, they are expressed as a range.

Table 4.42. LOQ (ng/l) for polybrominated diphenylethers in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	0.05 - 0.36				0.15			
octaBDE	0.56 - 2.41				0.15			
decaBDE	5.62 - 5.98				0.15			1

Altogether 105 municipal effluent samples were analysed for PBDEs (Table 4.43.). PentaBDE, octaBDE and decaBDE were observed in 36 %, 2% and 65% of samples, respectively. The highest concentrations of pentaBDE (0.90 ng/l) and octaBDE (1.5 ng/l) were observed in Finland. The highest decaBDE concentration was found in Denmark (23 ng/l) (Figure 4.36.).

Table 4.43. Number of samples and observed frequencies of PBDE concentrations above LOQ in municipal effluents.

	Number of samples	pentaBDE	octaBDE	decaBDE
Denmark	6	5	0	2
Estonia	12	5	0	11
Finland	18	9	1	13
Germany	12	5	0	10
Latvia	4	2	1	4
Lithuania	11	4	0	10
Poland	18	8	0	18
Sweden	24	0	0	0
TOTAL	105	38	2	68
%		36	2	65

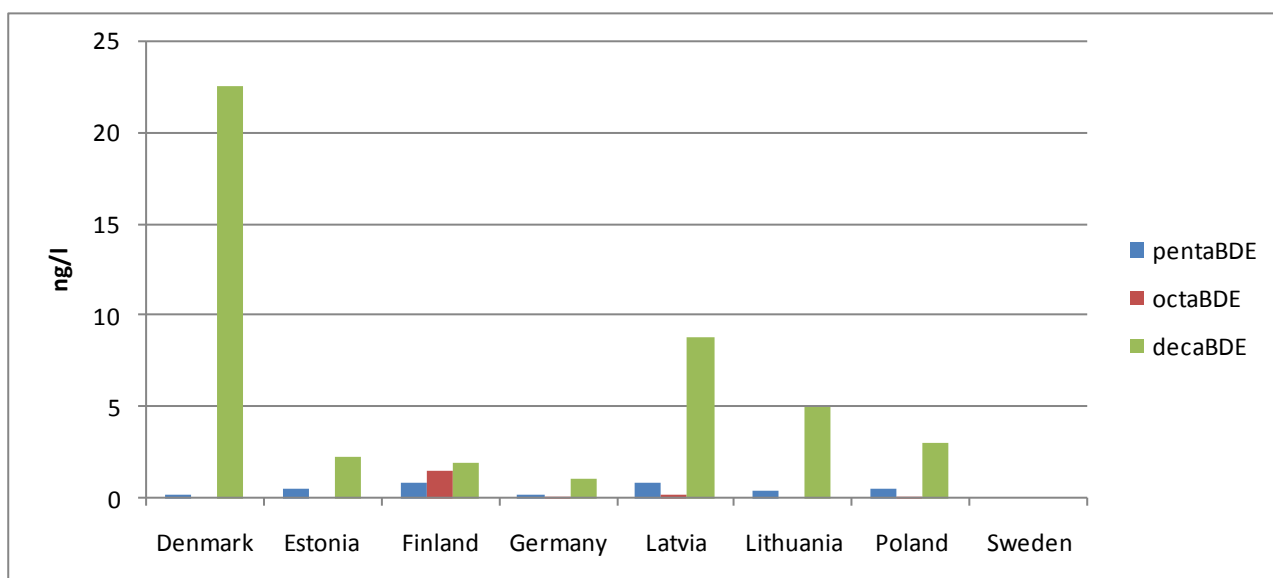


Figure 4.36. The highest concentrations (ng/l) of PBDEs found in municipal effluents.

In municipal effluents decaBDE was the most frequently found congener and its concentration was also highest. In Sweden PBDEs were not found, but it has to be bear in mind that results are presented as sum parameters and LOQ was higher.

Industrial effluents

LOQs varied between laboratories and substances (Table 4.44.). If LOQs varied between the congeners, they are expressed as a range.

Table 4.44. LOQs (ng/l) of PBDEs in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	
pentaBDE	0.05 - 0.34	0.15						
octaBDE	0.05 - 2.29	0.15						
decaBDE	5.66 - 6.72	0.15						

PDEs were analysed from 53 industrial effluent samples (Table 4.45.). PentaBDE, octaBDE and decaBDE were observed in 21 %, 6% and 68% of samples, respectively. The highest concentration of pentaBDE, octaBDE and decaBDE were found from Lithuanian samples (4.5 ng/l, 14 ng/l and 10 ng/l, respectively) (Figure 4.37.).

Table 4.45. Number of samples and frequencies of PBDE concentrations above LOQ in industrial effluents.

	Number of samples	pentaBDE	octaBDE	decaBDE
Denmark	4	2	1	1
Estonia	12	4	0	12
Finland	6	0	0	5
Germany	10	0	0	3
Latvia	4	1	1	3
Lithuania	11	3	1	8
Poland	6	1	0	4
Sweden	0	-	-	-
TOTAL	53	11	3	36
%		21	6	68

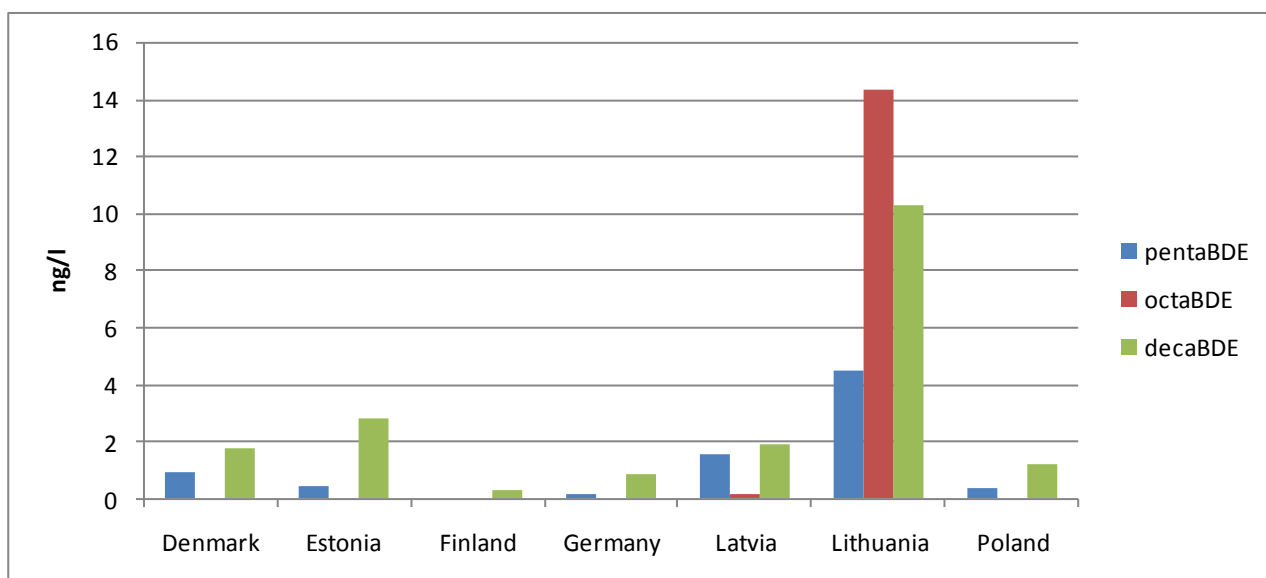


Figure 4.37. The highest concentrations (ng/l) of PBDEs found in industrial effluents.

In industrial effluents decaBDE was most frequently observed, but the highest concentration was found for octaBDE. OctaBDE was found only in Latvia and Lithuania.

Storm water

LOQ are given in table 4.46. If LOQs varied between the congeners, they are expressed as a range.

Table 4.46. LOQs (ng/l) of PBDEs in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	0.03 - 0.35			0.15				0.5
octaBDE	0.17 - 2.33			0.15				0.1
decaBDE	3.3			0.15				1

Fifteen storm water samples were analysed for PBDEs (Table 4.47.). PentaBDE, octaBDE and decaBDE were observed in 60 %, 20% and 67% of samples, respectively. The highest concentration was found for pentaBDE in Estonia (1.3 ng/l) and for octaBDE (1.1 ng/l) and decaBDE (10 ng/l) in Denmark (Figure 4.38.).

Table 4.47. Number of samples and frequencies of PBDE concentrations above LOQ in storm water.

	Number of samples	pentaBDE	octaBDE	decaBDE
Denmark	2	2	1	1
Estonia	2	1	0	2
Finland	2	1	0	2
Germany	2	2	1	2
Latvia	1	1	0	1
Lithuania	2	0	0	1
Poland	2	1	1	1
Sweden	2	1	0	0
TOTAL	15	9	3	10
%		60	20	67

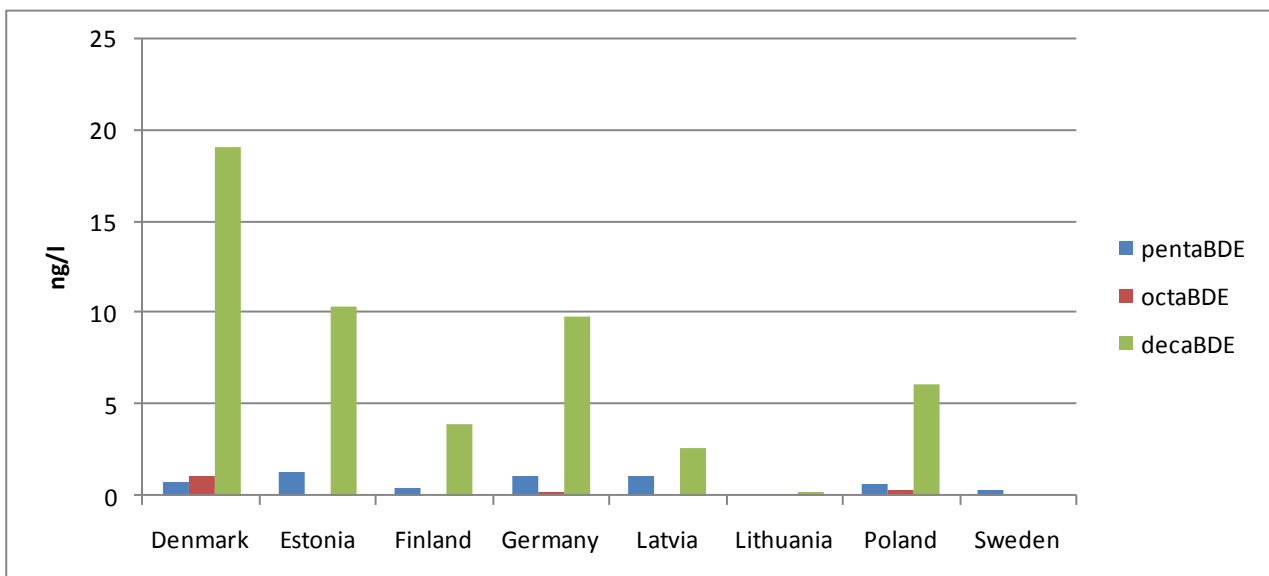


Figure 4.38. The highest concentrations (ng/l) of PBDEs found in storm water.

Landfill leachate

LOQ values are presented in table 4.48. If LOQs varied between the congeners, they are expressed as a range.

Table 4.48. LOQs (ng/l) of PBDEs in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	0.06 - 0.50			0.15				
octaBDE	0.1 - 2.99			0.15				
decaBDE	2.74			0.15				

Fifteen landfill leachate samples were analysed for PBDEs (Table 4.49.). PentaBDE, octaBDE and decaBDE were found in 67 %, 40% and 67% of samples, respectively. The highest concentration was recorded for pentaBDE and octaBDE in Poland (32 ng/l and 2.7 ng/l, respectively) and for decaBDE in Denmark (41 ng/l) (Figure 4.39.).

Table 4.49. Number of samples and frequencies of PBDE concentrations above LOQ in landfill leachate.

	Number of samples	pentaBDE	octaBDE	decaBDE
Denmark	2	1	1	1
Estonia	2	2	1	2
Finland	2	2	2	2
Germany	2	2	1	2
Latvia	1	1	0	1
Lithuania	2	0	0	0
Poland	2	2	1	2
Sweden	2			
TOTAL	15	10	6	10
%		67	40	67

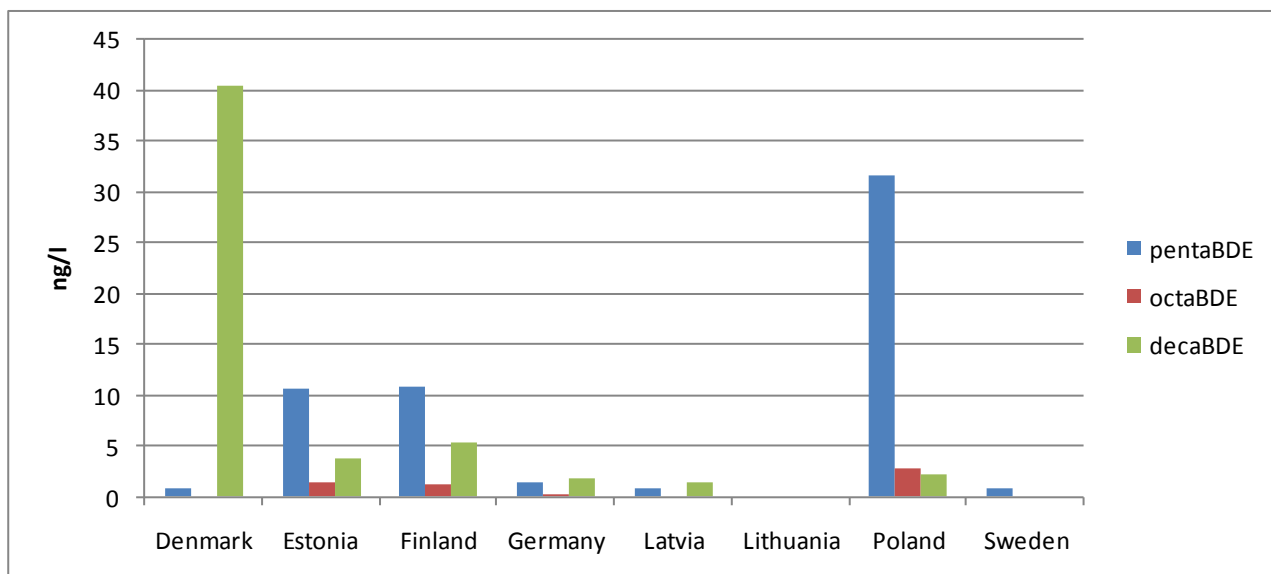


Figure 4.39. The highest concentrations (ng/l) of PBDEs found in landfill leachate.

In landfill leachate octaBDE and decaBDE was equally frequently found. The highest concentration was found for decaBDE. In Lithuania none of the PBDEs were found.

Analytical methods for sludge

In SYKE freeze-dried sample (1 g) was extracted with dichloromethane (DCM) at pressurized liquid extraction. Surrogate standard ($^{13}\text{C}_{12}$ -BDE 77) was added to the samples before extraction. The extracts were cleaned with multilayer silica column and basic alumina column (according to standard ISO 22032). The samples were concentrated and solvent was changed to nonane before instrumental analysis. Low pressure gas chromatography tandem mass spectrometry (LP-GC-TQ MS) was used for instrumental analysis of PBDE compounds. Prior to injection, $^{13}\text{C}_{12}$ -BDEs (28, 47, 99, 153, 183, 209) were added as quantification standards. The final results are recovery corrected and the blank samples were determined in the all sample series.

For the Danish samples Eurofins GfA GmbH in Germany added an internal $^{13}\text{C}_{12}$ marked PBDE standard ($^{13}\text{C}_{12}$ -TriBDE, $^{13}\text{C}_{12}$ -TetraBDE, $^{13}\text{C}_{12}$ -PentaBDE, $^{13}\text{C}_{12}$ -HexaBDE, $^{13}\text{C}_{12}$ -HeptaBDE, $^{13}\text{C}_{12}$ -DecaBDE) to the extracts and the extracts were cleaned by column chromatography. Analysis was performed by means of capillary gas chromatography coupled with mass spectrometry (GC/MS). Quantification of the native PBDEs via the internal ^{13}C marked standard.

Sludge

LOQ values are presented in table 4.50. If LOQs varied between the congeners, they are expressed as a range.

Table 4.50. LOQs ($\mu\text{g}/\text{kg}$) of PBDEs in sludge (n.i. not informed).

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	0.05 - 0.36	0.02 - 0.1						n.i.
octaBDE	0.56 - 2.41	0.01 - 0.06						n.i.
decaBDE	5.62 - 5.98	0.32						n.i.

In sludge pentaBDE, octaBDE and decaBDE were found in 91 % of samples (22 samples, Table 4.51.). The highest concentration was recorded for pentaBDE in Finland (62 $\mu\text{g}/\text{kg}$), and for decaBDE in Estonia (1200 $\mu\text{g}/\text{kg}$, Figure 4.40.). OctaBDE concentration was 2.5 $\mu\text{g}/\text{kg}$ in Denmark, Finland and Latvia.

Table 4.51. Number of samples and frequencies of PBDE concentrations above LOQ in sludge (n.i. not informed).

	Number of samples	pentaBDE	octaBDE	decaBDE
Denmark	4	4	4	4
Estonia	3	3	3	3
Finland	2	2	2	2
Germany	3	3	3	3
Latvia	4	4	4	4
Lithuania	2	2	2	2
Poland	2	2	2	2
Sweden	2	n.i.	n.i.	n.i.
TOTAL	22	20	20	20
%		91	91	91

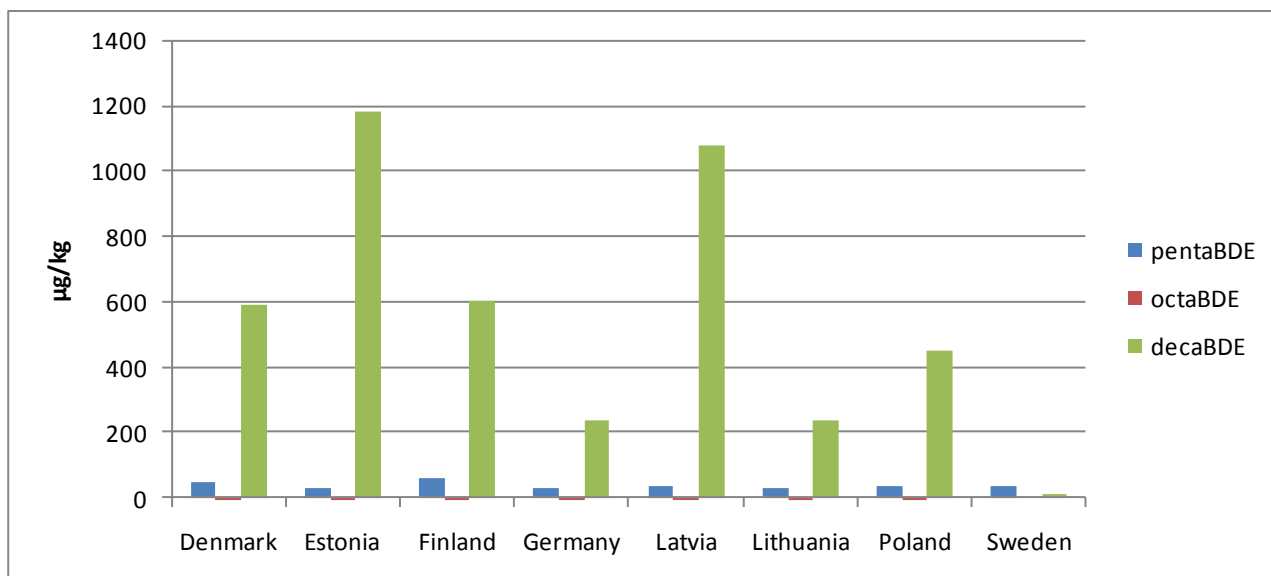


Figure 4.40. The highest concentrations ($\mu\text{g}/\text{kg}$) of PBDEs in sludge.

In sludge samples pentaBDE, octaBDE and decaBDE was found equally often. The highest concentrations were found for decaBDE. Penta- and decaBDE were found in every country.

4.7.2 Hexabromocyclododecanes, HBCDs

Technical hexabromocyclododecane is used as a flame retardant in extruded and expanded polystyrene foam used as thermal insulation in the building industry. HBCDs are brominated cycloalkanes with 16 possible stereoisomers. The isomers have different chemical properties and biological effects. HBCDs are thermolabile compounds and thermal rearrangement of isomers occurs at temperatures above 160°C leading to changes of isomer ratios. Therefore, in instrumental analysis it is important not to affect isomer ratios.

Currently HBCDs are under review of Stockholm Convention. HBCD is mentioned in BSAP as substances of specific concern to the Baltic Sea. Technical HBCD contains mainly γ -isomer, but α - and β -isomers are also present. Although γ -isomer predominates in products, α -isomer is the main isomer found in aquatic organisms and sediments.

Finland (SYKE) analysed HBCDs for Estonia, Germany, Latvia, Lithuania, Poland and Finland. Sweden (IVL) and Denmark (Eurofins) analysed their own samples.

Analytical method for effluents, storm water and landfill leachate

In SYKE water samples (approx. 1600 ml) were extracted as whole samples. The liquid-liquid extraction with DCM was performed twice to assure good recoveries (over 70%). The extracts were cleaned with basic alumina column. The samples were concentrated and solvent was changed to methanol before instrumental analysis. Ultra performance liquid chromatography (UPLC) tandem mass spectrometry (TQ MS/MS) was used for instrumental analysis of three HBCD diastereomers

(α -, β - and γ -HBCD). Isotopic dilution technique using ^{13}C - and d_{18} -labelled HBCD isomers as internal standards was applied for quantification and recovery calculations of each diastereomer. The final results are recovery corrected. A blank sample was analysed in each sample batch.

In IVL water samples were filtrated. The filtrates were purified and concentrated by SPE (C18 column) and filter was extracted with acetone and hexane:MTBE. Extracts were combined and clean-up was done by sulphuric acid and silica gel column. The BDE-congeners 119, 166 and 190 were used as internal standard.

In Eurofins water samples were extracted as whole samples (800 ml). The internal standards (^{13}C -labelled γ -HBCD) were added before liquid-liquid extraction with toluene was performed three times. The extracts were purified with sulphuric acid and basic alumina column. The extracts were concentrated (50 μl) and recovery standard (^{13}C -labelled BDE138) was added before instrumental analysis. Gas chromatography with low resolution mass spectrometry (HRGC/LRMS) was used for instrumental analysis of the sum of HBCD. The final results are recovery corrected. At least one blank is included.

The results of HBCDs were not comparable between the laboratories. Variations in the results are at least partly derived from the different methods: 1) Two laboratories analysed sum of all HBCD compounds using a technical HBCD mixture in calibration and standard solution. One laboratory analysed individual isomers of HBCD and used the mixture of pure isomers in calibration and standard solutions. 2) Two laboratories used BDE-congeners as internal standard and one laboratory used mass labelled (^{13}C and d_{18}) isomers. 3) One laboratory applied GC-ECD method, the other laboratory GC-LRMS and third one LC-MS/MS method for analysis of HBCDs.

Municipal effluents

LOQ values are shown in table 4.52. In frequency studies, in case where isomers are analysed separately the SUM HBCD is considered to be above LOQ when at least one of the isomers is above the LOQ. When LOQ had changed during the project the LOQ is expressed as a range.

Table 4.52. LOQs (ng/l) of HBCDs in municipal effluent.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -HBCD	-			0.1				-
β -HBCD	-			0.1				-
γ -HBCD	-			0.1				-
SUM HBCD	1.13 - 5.0			-				0.1 - 1

Individual HBCD isomers were analysed from 76 municipal effluent samples and the sum HBCD from 130 samples (Table 4.53.).

α -HBCD, β -HBCD and γ -HBCD were observed in 79 %, 39 % and 70 % of municipal effluent samples, respectively. The highest concentrations of α -HBCD, β -HBCD and γ -HBCD were found in Germany (8.3 ng/l, 4.1 ng/l and 56 ng/l, respectively, Figure 4.41.). Concentrations above the detection of sum HBCD were found in 52 % of the samples and the highest concentration (68 ng/l) was found in Germany.

Table 4.53. Number of samples and frequencies of HBCD concentrations above LOQ in municipal effluents.

	Number of samples	α -HBCD	β -HBCD	γ -HBCD	No of samples	SUM HBCD
Denmark	0	-	-	-	6	1
Estonia	12	11	5	8	12	11
Finland	18	13	7	12	18	13
Germany	12	5	1	8	12	8
Latvia	4	4	0	4	4	4
Lithuania	12	9	6	10	36	10
Poland	18	18	11	11	18	18
Sweden	0	-	-	-	24	3
TOTAL	76	60	30	53	130	68
%		79	39	70		52

- not analysed

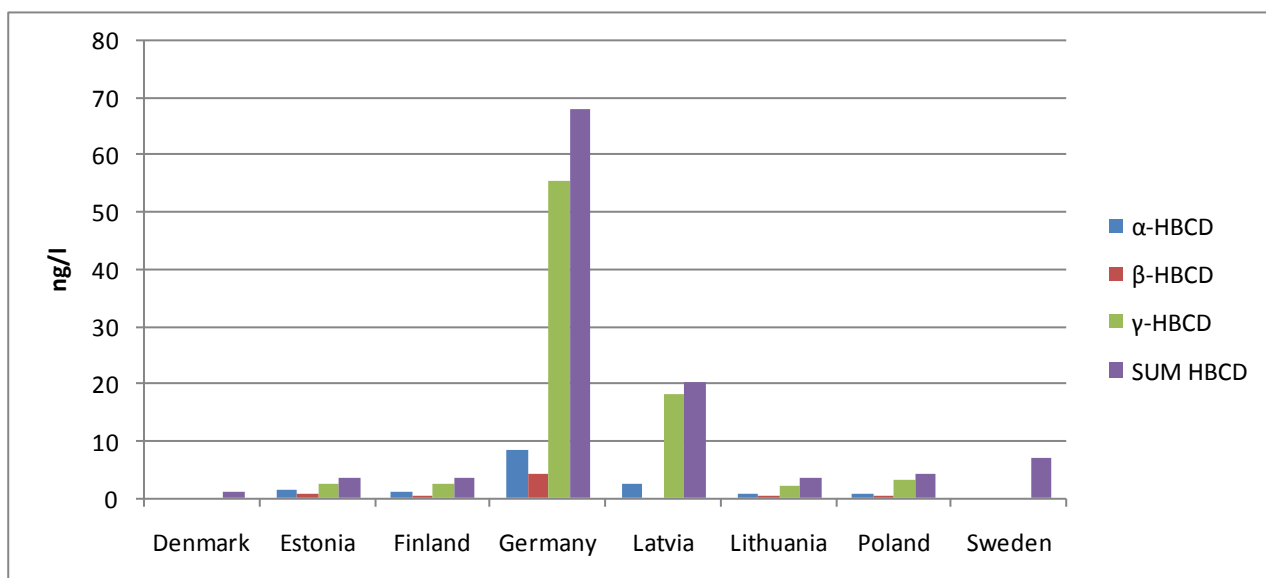


Figure 4.41. The highest concentrations (ng/l) of HBCDs found in municipal effluents.

In municipal effluent α -HBCD was most frequently found, but the highest concentrations were found for γ -HBCD. Both α - and γ -HBCD were found in every country.

Industrial effluents

LOQ values are given in table 4.54. In case where isomers were analysed separately, the SUM HBCD was considered to be above LOQ, when at least one of the isomers was above the LOQ.

Table 4.54. LOQs (ng/l) of HBCDs in effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland
α -HBCD	-				0.1		
β -HBCD	-				0.1		
γ -HBCD	-				0.1		
SUM HBCD	0.01 - 5.0				-		

Individual HBCD isomers were analysed from 50 industrial effluent samples and the sum HBCD from 78 samples (Table 4.55.). α -HBCD, β -HBCD and γ -HBCD were observed in 54 %, 22 % and 60 % of the samples, respectively. The highest concentrations of α -HBCD, β -HBCD and γ -HBCD were found in Lithuania (32 ng/l, 7.3ng/l and 73 ng/l, respectively, Figure 4.42.). Sum HBCD was observed in 63 % of industrial effluent samples. The maximum concentration (110 ng/l) was found in Lithuania.

In industrial effluent γ -HBCD was most frequently found and its concentration was also the highest. Both α - and γ -HBCD were found in every country.

Table 4.55. Number of samples and frequencies of HBCD concentrations above LOQ in industrial effluents.

	Number of samples	α -HBCD	β -HBCD	γ -HBCD	Number of samples	SUM HBCD
Denmark	0	-	-	-	4	0
Estonia	12	11	5	8	12	11
Finland	6	3	2	4	6	4
Germany	11	4	0	5	11	5
Latvia	3	1	0	2	3	2
Lithuania	12	7	3	8	12	9
Poland	6	1	1	3	6	3
Sweden	0	-	-	-	0	-
TOTAL	50	27	11	30	54	34
%		54	22	60		63

- not analysed

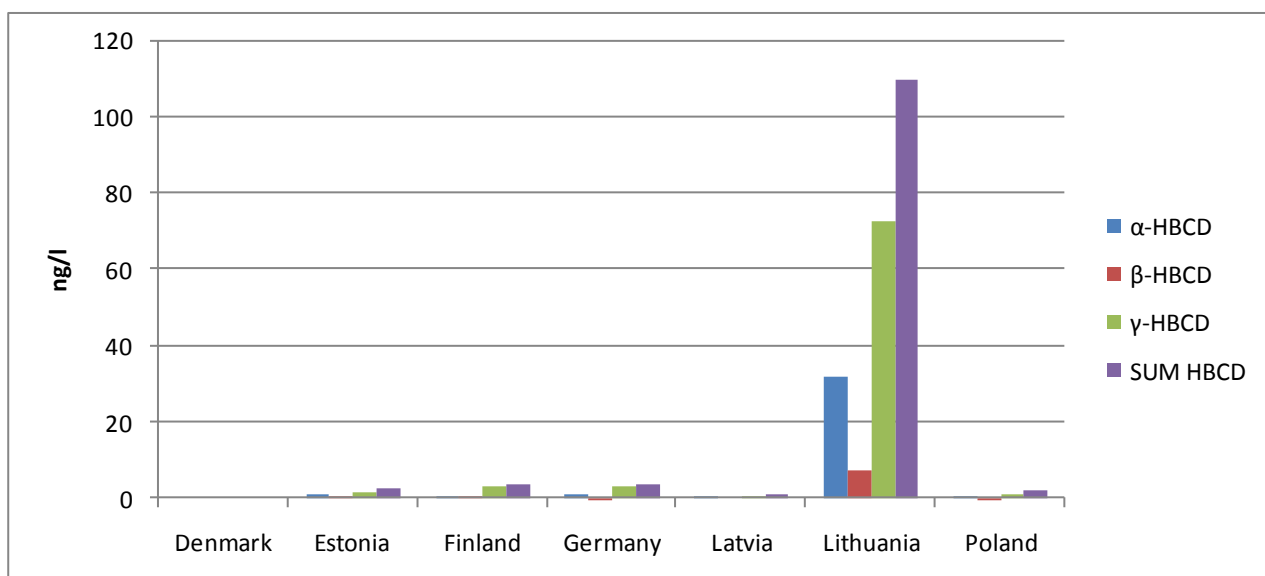


Figure 4.42. The highest concentrations (ng/l) of HBCDs found in industrial effluents.

Storm water

LOQ values are given in table 4.56. When isomers were analysed separately, the sum HBCD was considered to be above LOQ, when at least one of the isomers were above the LOQ.

Table 4.56. LOQs (ng/l) of HBCDs in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α-HBCD	-			0.1				-
β-HBCD	-			0.1				-
γ-HBCD	-			0.1				-
SUM HBCD	3.0 - 5.0			-				1

Eleven storm water samples were analysed for individual HBCD isomers and 19 for sum HBCD (Table 4.57.). α-HBCD, β-HBCD and γ-HBCD were observed in 64 %, 45 % and 73 % of the samples, respectively. The highest concentration for α-HBCD, β-HBCD and γ-HBCD was found in Germany (2.9 ng/l, 1.3 ng/l and 68 ng/l, respectively, Figure 4.43.). Sum HBCD was found in 39 % of storm water samples.

Table 4.57. Number of samples and frequencies of HBCD concentrations above LOQ in storm water samples.

	Number of samples	α -HBCD	β -HBCD	γ -HBCD	Number of samples	SUM HBCD
Denmark	0	-	-	-	2	0
Estonia	2	1	1	2	2	2
Finland	2	2	0	1	2	2
Germany	2	1	2	2	2	2
Latvia	1	1	1	1	1	1
Lithuania	2	1	1	1	3	1
Poland	2	1	0	1	2	1
Sweden	0	-	-	-	2	1
TOTAL	11	7	5	8	16	10
%		64	45	73		39

- not analysed

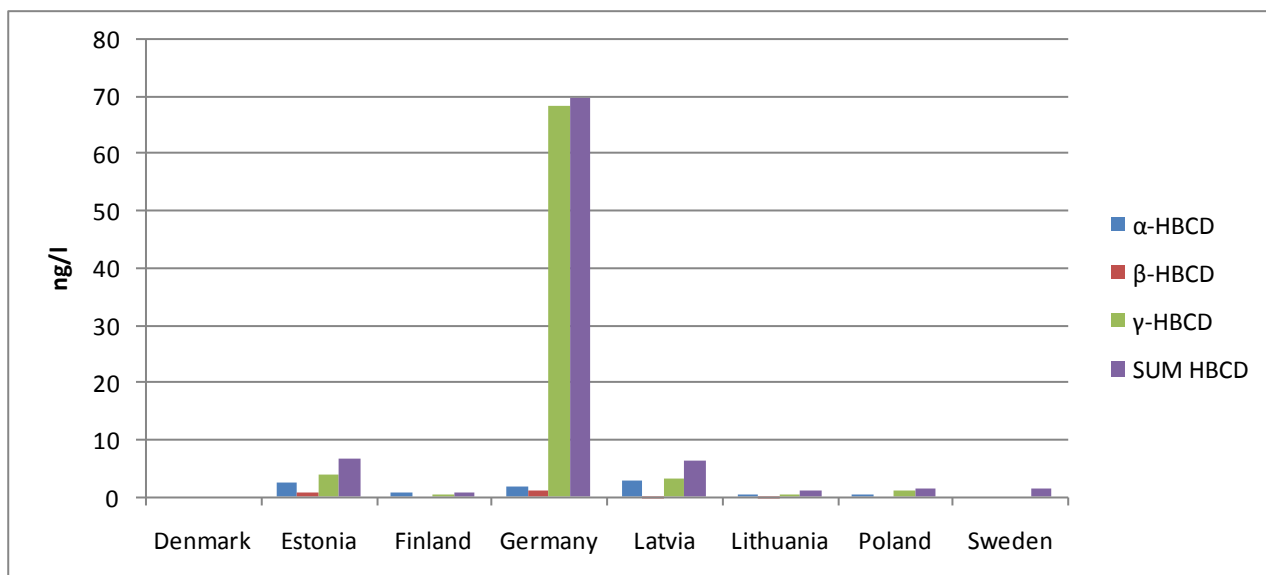


Figure 4.43. The highest concentrations (ng/l) of HBCDs found in storm water.

In storm water γ -HBCD was most frequently found and its concentration was highest. Both α - and γ -HBCD were observed in every country.

Landfill leachate

LOQ values are given in table 4.58. When isomers were analysed separately, the SUM HBCD was considered to be above LOQ, when at least one of the isomers is above the LOQ.

Table 4.58. LOQ (ng/l) of HBCDs in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -HBCD	-	0.1						-
β -HBCD	-	0.1						-
γ -HBCD	-	0.1						-
SUM HBCD	3.0 - 4.55	-						1

In landfill leachate α -HBCD, β -HBCD and γ -HBCD were noted in 18 %, 9 % and 36 % of samples, respectively (11 analysed samples, Table 4.59.). The maximum concentration was found for α -HBCD, β -HBCD and γ -HBCD in Latvia (8.1 ng/l, 55 ng/l and 12 ng/l, respectively). Sum HBCD was observed in 25 % of landfill leachate samples (19 analysed samples). The maximum concentration (75 ng/l) was found in Latvia (Figure 4.44.).

In landfill leachate γ -HBCD was most frequently found, but the highest concentration was found for β -HBCD. α - and β -HBCD were found only in Estonia and Latvia. In Lithuania none of HBCD isomers were found.

Table 4.59. Number of samples and frequencies of HBCD concentrations above LOQ in landfill leachate.

	Number of samples	α -HBCD	β -HBCD	γ -HBCD	Number of samples	SUM HBCD
Denmark	0	-	-	-	2	0
Estonia	2	1	0	0	2	1
Finland	2	0	0	1	2	1
Germany	2	0	0	1	2	1
Latvia	1	1	1	1	1	1
Lithuania	2	0	0	0	2	0
Poland	2	0	0	1	2	0
Sweden	0	-	-	-	2	0
TOTAL	11	2	1	4	16	4
%		18	9	36		25

- not analysed

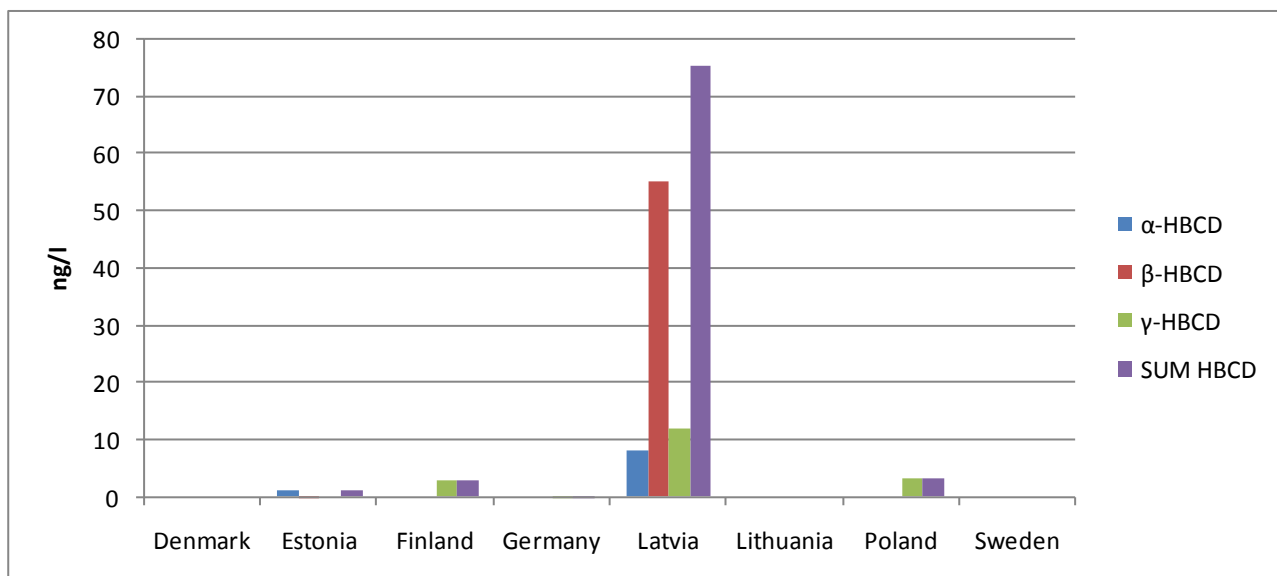


Figure 4.44. The highest concentrations (ng/l) of HBCDs found in landfill leachate.

Analytical methods for sludge

In SYKE freeze-dried sample (1 g) was extracted with DCM at pressurized liquid extraction. Surrogate standards ($^{13}\text{C}_{12}$ - α -, β - and γ -HBCD) were added to the samples before extraction. The extracts were cleaned with acidified silica and basic alumina columns. The samples were concentrated and solvent was changed to methanol before instrumental analysis. Ultra performance liquid chromatography (UPLC) tandem mass spectrometry (TQ MS) was used for instrumental analysis of three HBCD diastereomers (α -, β - and γ -HBCD). Prior to injection, d_{18} α -, β - and γ -HBCD were added as quantification standards. The final results are recovery corrected and the blank samples were determined in the all sample series. Description of the Swedish method was not available.

In Denmark Eurofins added the internal $^{13}\text{C}_{12}$ marked γ -HBCD standard to the extracts. Clean-up of the extract was done by column chromatography. Analysis was performed by means of capillary gas chromatography coupled with mass spectrometry (GC/MS), identification via molecule or fragmentations and quantification of the total native HBCD isomers via the internal ^{13}C marked standard (isotope dilution method).

Sludge

LOQ values are given in table 4.60. When isomers were analysed separately, the sum HBCD was considered to be above LOQ, when at least one of the isomers was above the LOQ.

Fourteen sludge samples were analysed for HBCD isomers and 20 for sum HBCD (Table 4.61.). α -HBCD was found in all sludge samples, β -HBCD in 64 % and γ -HBCD in 93 % of the samples. The highest concentration was found for α -HBCD and β -HBCD in Estonia (97 $\mu\text{g}/\text{kg}$ and 14 $\mu\text{g}/\text{kg}$, respectively) and for γ -HBCD in Latvia (200 $\mu\text{g}/\text{kg}$) (Figure 4.45.). Sum HBCD was observed in 85 % of sludge samples. The maximum concentration (210 $\mu\text{g}/\text{kg}$) was found in Latvia.

Table 4.61. LOQs ($\mu\text{g}/\text{kg}$) of HBCDs in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -HBCD	-	0.5						-
β -HBCD	-	0.5						-
γ -HBCD	-	0.5						-
SUM HBCD	0.5 - 1.0	-						1

Table 4.61. Number of samples and frequencies of HBCD concentrations above LOQ in sludge.

	Number of samples	α -HBCD	β -HBCD	γ -HBCD	Number of samples	SUM HBCD
Denmark	0	-	-	-	4	2
Estonia	3	3	1	3	3	3
Finland	2	2	1	2	2	2
Germany	3	3	2	3	3	3
Latvia	2	2	2	2	2	2
Lithuania	2	2	2	2	2	2
Poland	2	2	1	1	2	2
Sweden	0	-	-	-	2	1
TOTAL	14	14	9	13	20	17
%		100	64	93		85

- not analysed

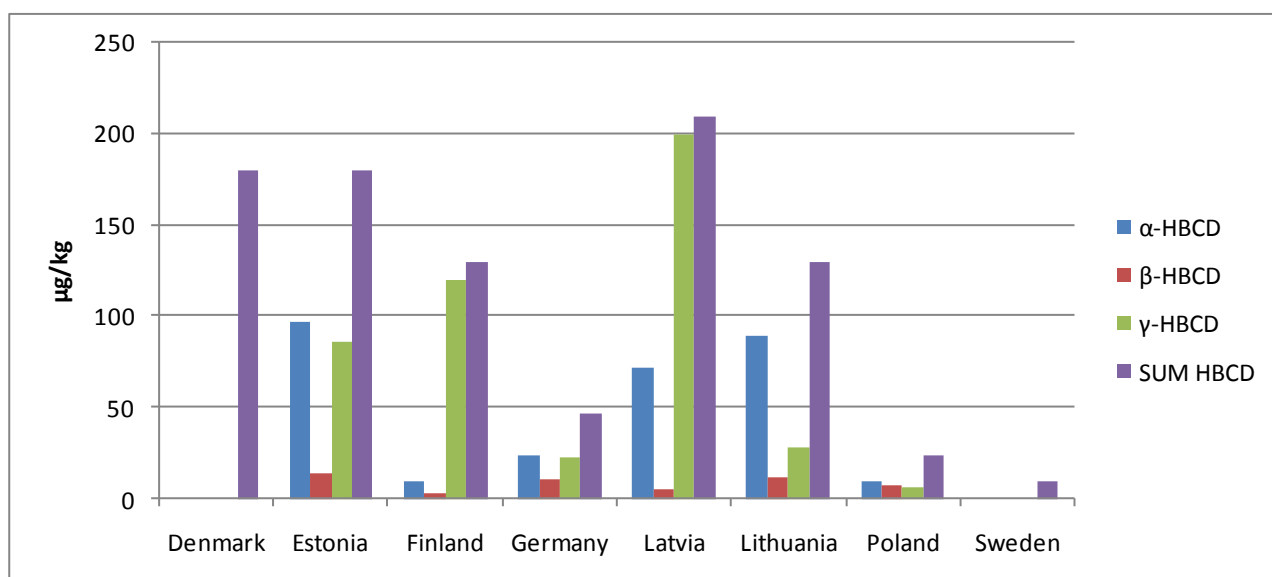


Figure 4.45. The highest concentrations ($\mu\text{g}/\text{kg}$) of HBCDs found in sludge.

In sludge α -HBCD was most frequently found, but the highest concentrations were observed for γ -HBCD. All HBCD isomers were found in every country.

4.8 Perfluorinated compounds

Perfluoroalkyl acids are a group of fully fluorinated substances with different acidic groups (e.g. carboxylic or sulfonic acid). Perfluorooctane sulphonic acid (PFOS) and perfluorooctanoic acid (PFOA) are mentioned in BSAP as substances of specific concern to the Baltic Sea.

PFOA and PFOS are chemically and biologically inert and very stable. Perfluoroalkyl acids are used widespread *e.g.* in electric and electronic parts, fire fighting foam, photo imaging, hydraulic fluids and textiles. PFOS, its salts and perfluorooctane sulfonyl fluoride are included in the list of Stockholm Convention on persistent organic pollutants. Their production and use is restricted. The marketing and use of perfluorooctane sulfonates is restricted in Europe.

Finland (SYKE) analysed perfluoroalkyl acids for Estonia, Germany, Latvia, Lithuania, Poland and Finland. Sweden (IVL) and Denmark (Eurofins) analysed their own samples. PFOS, PFOA, perfluoro-*n*-hexanoic acid (PFHxA) and perfluoro-*n*-decanoic acid (PFDA) were analysed.

Analytical method for effluents, storm water and landfill leachate

The analytical methods for effluents, storm water and landfill leachate used in analysing laboratories were quite similar.

In SYKE the surrogate standards ($^{13}\text{C}_2$ -PFHxA, $^{13}\text{C}_4$ -PFOS and $^{13}\text{C}_2$ -PFDA) were added to the samples (250 ml) before centrifugation. Supernatant was purified and concentrated using solid phase extraction (SPE C18 cartridges). A portion of the extract was analysed without concentration. Prior to injection, $^{13}\text{C}_4$ -PFOA was added as a recovery standard. Instrumental analysis was performed with LC-MS/MS using water and methanol buffered with ammonium acetate as LC effluents. Calibration was done using linear-only standards and the results were reported as a sum of both linear and branched isomers. The final results are recovery corrected. A blank sample was included in each batch of samples.

In IVL standards ($^{13}\text{C}_4$ -PFOS and $^{13}\text{C}_4$ -PFOA) were added to the samples (300 ml) before purification and concentration by SPE (C18 cartridges). The extract was concentrated with nitrogen to 1 ml. Instrumental analysis was performed with LC-MS/MS using water and methanol buffered with ammonium acetate as LC effluents.

In Eurofins surrogate standards ($^{13}\text{C}_2$ -PFHxA, $^{13}\text{C}_8$ -PFOA, $^{13}\text{C}_5$ -PFNA, $^{13}\text{C}_2$ -PFDA and $^{13}\text{C}_4$ -PFOS) were added to the samples (50 ml) before SPE (weak anion exchange cartridges). The extract was evaporated with nitrogen to dryness, and then redissolved with methanol/water (1:1). The final volume was 100 μl containing $^{13}\text{C}_4$ -PFOA as a recovery standard. Instrumental analysis was performed with LC-MS/MS using water and methanol buffered with ammonium acetate as LC effluents. The calibration was done using linear-only standards and the results were reported as a sum of both linear and branched isomers. The final results are recovery corrected. A minimum of two blanks and a reference sample were included in each batch of samples.

Municipal effluents

LOQs varied between laboratories and substances (Table 4.62.). When LOQ had changed during the project, it is expressed as a range.

Table 4.62. LOQ (ng/l) for perfluoroalkyl acids in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	3.3 - 5.0	0.5						0.5
PFOA	3.3 - 5.0	0.5						0.5
PFHxA	3.3 - 5.0	0.5						0.5 - 12
PFDA	3.3 - 5.0	0.5						0.5 - 0.7

In total 106 municipal effluent samples were analysed for perfluoroalkyl acids (Table 4.63.). PFOS, PFOA, PFHxA and PFDA were observed in 86%, 98%, 76% and 58% of the samples, respectively (Table 4.63.). The highest perfluoroalkyl acid concentrations were found in Finland PFOS 640 ng/l, in Poland and Sweden PFOA 18 ng/l, in Sweden PFHxA 10 ng/l and in Denmark PFDA 5.7 ng/l (Figure 4.46.).

Table 3.9.2. Number of samples and frequencies of perfluoroalkyl acid concentrations above LOQ in municipal effluents.

	Number of samples	PFOS	PFOA	PFHxA	PFDA
Denmark	6	1	6	6	1
Estonia	12	8	12	4	5
Finland	18	18	18	18	13
Germany	12	11	12	12	7
Latvia	4	4	4	4	2
Lithuania	12	9	12	2	4
Poland	18	15	16	15	16
Sweden	24	24	24	20	13
TOTAL	106	91	104	81	61
%		86	98	76	58

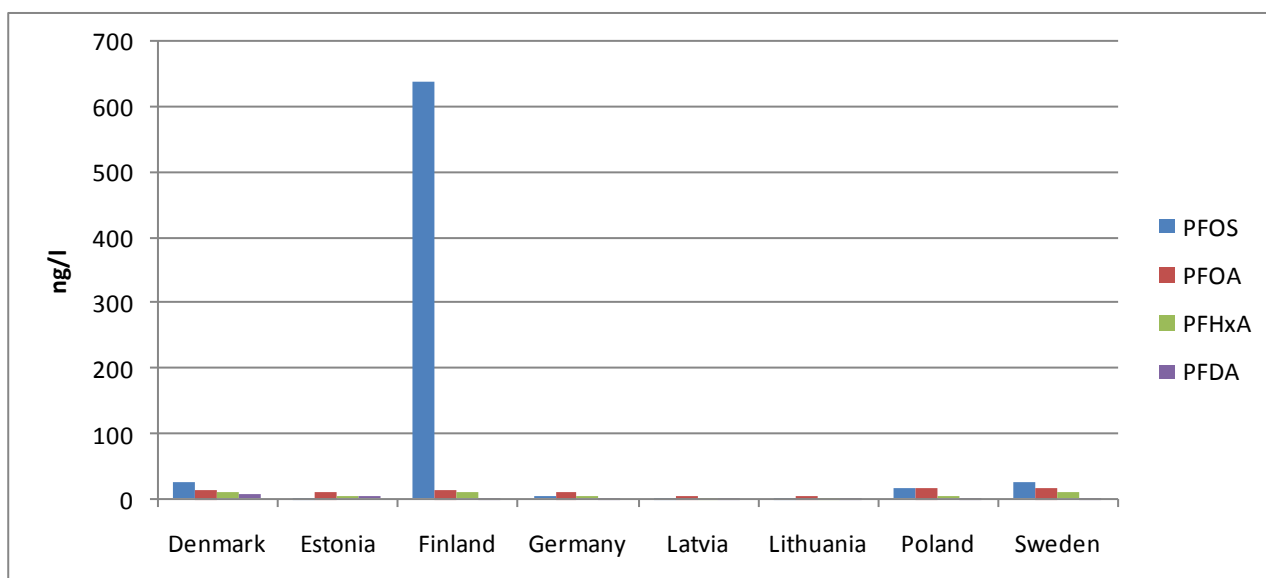


Figure 4.46. The highest concentrations (ng/l) of perfluoroalkyl acids in municipal effluents.

PFOA was the most frequently found perfluoroalkyl acid in municipal waste water effluent, but the highest concentrations were found for PFOS. All perfluoroalkyl acids were found in every country.

Industrial effluents

LOQ varied between laboratories and substances (Table 4.64.). After LOQ had changed during the project, it was expressed as a range.

Table 4.64. LOQs (ng/l) of perfluoroalkyl acids in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland
PFOS	3.3 - 5.0				0.5		
PFOA	2.0 - 10.0				0.5		
PFHxA	3.3 - 5.0				0.5		
PFDA	3.3 - 5.0				0.5		

In total 55 industrial effluent samples were collected for analysis (Table 4.65.). PFOS, PFOA, PFHxA and PFDA were observed in 62%, 95%, 58% and 15% of the samples, respectively. The maximum concentration was found for PFOS, PFOA, PFHxA and PFDA in Finland (1300 ng/l, 100 ng/l, 75 ng/l and 1.7 ng/l) (Figure 4.47.).

Table 4.65. Number of samples and frequencies of perfluoroalkyl acid concentrations above LOQ in industrial effluents.

	Number of samples	PFOS	PFOA	PFHxA	PFDA
Denmark	4	2	2	2	0
Estonia	12	11	12	9	0
Finland	6	6	6	6	5
Germany	11	6	11	5	1
Latvia	4	4	4	3	2
Lithuania	12	3	11	6	0
Poland	6	2	6	1	0
Sweden	0	-	-	-	-
TOTAL	55	34	52	32	8
%		62	95	58	15

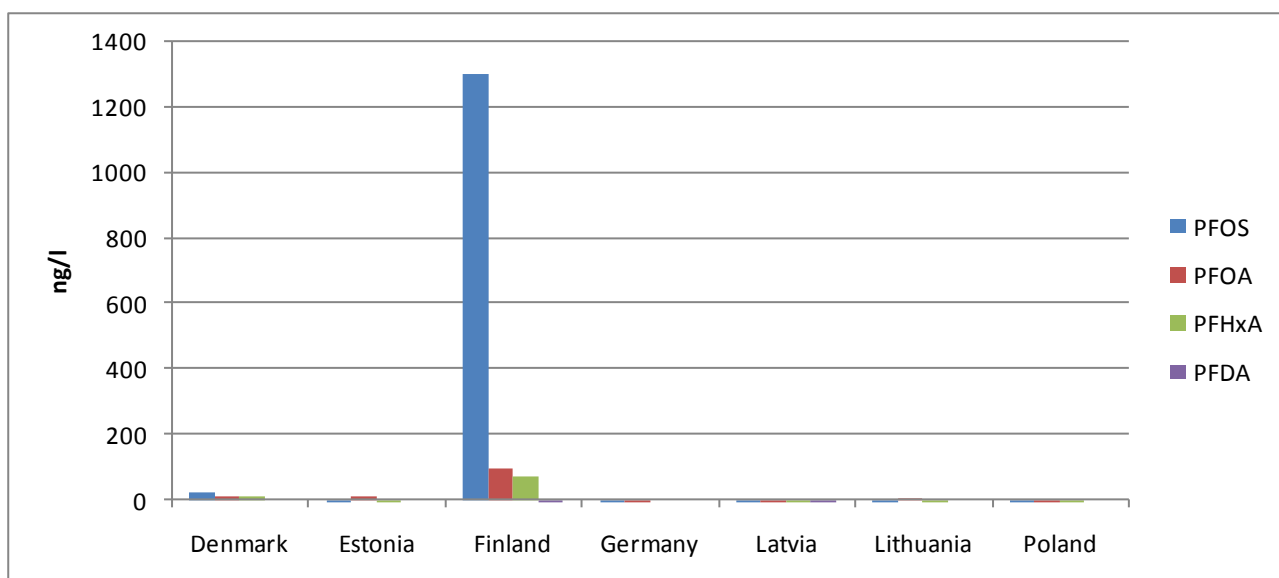


Figure 4.47. The highest concentrations (ng/l) of perfluoroalkyl acids in industrial effluents.

In industrial effluents PFOA was the most frequently found perfluoroalkyl acid, but the highest concentration was found for PFOS. PFOS, PFOA and PFHxA were found in every country. PFDA was found only in Finland, Germany and Latvia, but it has to bear in mind that in Denmark LOQ was higher than found concentrations.

Storm water

LOQ values are given in table 4.66.. The total number of storm water samples for perfluoroalkyl acid analyses was 15 (Table 4.67.). PFOS, PFOA, PFHxA and PFDA were observed in 87%, 87%, 53% and 40% of samples, respectively. The maximum concentration was observed for PFOS, PFOA and PFHxA in Denmark (420 ng/l, 67 ng/l and 180 ng/l) and for PFDA in Sweden (20 ng/l) (Figure 4.48.).

Table 4.66. LOQ (ng/l) for perfluoroalkyl acids in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	3.3				0.5			3.3
PFOA	3.3				0.5			3.3
PFHxA	3.3				0.5			3.3
PFDA	3.3				0.5			3.3

Table 4.67. Number of samples and observed frequencies of perfluoroalkyl acid concentrations above LOQ in storm water.

	Number of samples	PFOS	PFOA	PFHxA	PFDA
Denmark	2	1	2	1	1
Estonia	2	2	2	1	0
Finland	2	2	2	2	1
Germany	2	2	2	2	1
Latvia	1	1	0	0	0
Lithuania	2	2	2	0	1
Poland	2	1	1	0	0
Sweden	2	2	2	2	2
TOTAL	15	13	13	8	6
%		87	87	53	40

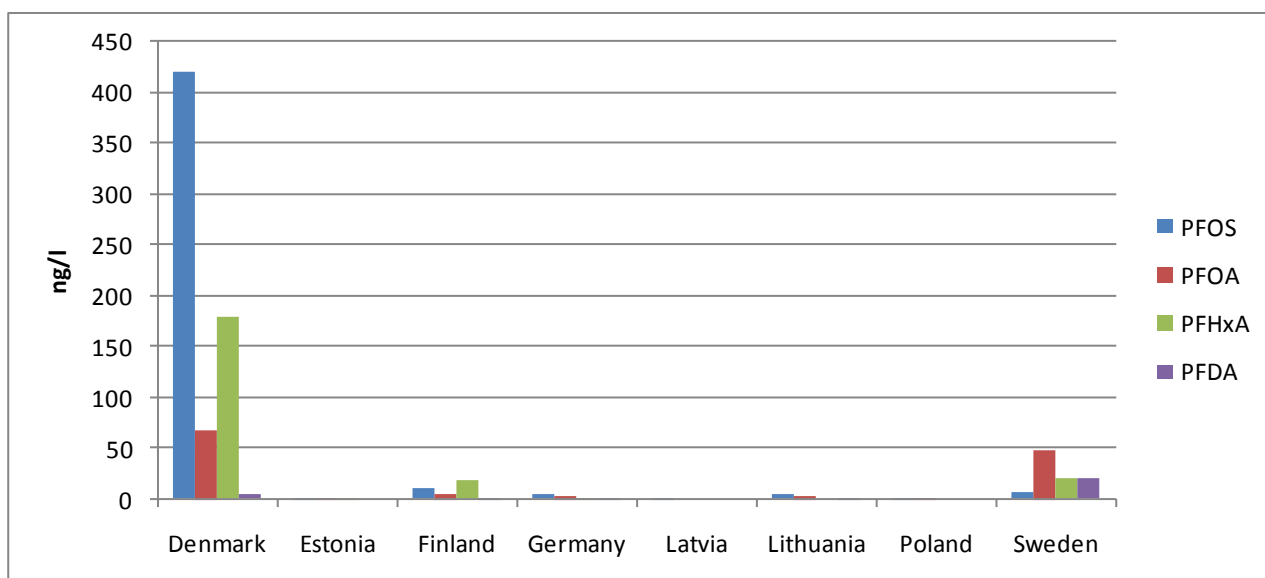


Figure 4.48. The highest concentrations (ng/l) of perfluoroalkyl acids in storm water samples.

In storm water PFOA was most frequently found perfluoroalkyl acid, but PFOS was at the highest concentration and was found in every country.

Landfill leachate

LOQ values are given in table 4.68. In total 15 landfill leachate samples were collected for analysis of perfluoroalkyl acids (Table 4.69.). PFOS, PFOA, PFHxA and PFDA were found in 73%, 93%, 87% and 73% of the samples, respectively. The maximum concentration was observed for PFOS, PFOA and PFHxA in Sweden (1500 ng/l, 2000 ng/l and 2900 ng/l) and for PFDA in Poland (200 ng/l) (Figure 4.49.).

Table 4.68. LOQ (ng/l) for perfluoroalkyl acids in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	10				0.5			0.5
PFOA	10				0.5			0.5
PFHxA	10				0.5			0.5
PFDA	10				0.5			0.5

Table 4.69. The number of samples and frequencies of perfluoroalkyl acid concentrations above LOQ in landfill leachate samples.

	Number of samples	PFOS	PFOA	PFHxA	PFDA
Denmark	2	0	2	2	0
Estonia	2	2	2	2	2
Finland	2	2	2	2	2
Germany	2	2	2	2	2
Latvia	1	1	1	1	1
Lithuania	2	0	1	0	0
Poland	2	2	2	2	2
Sweden	2	2	2	2	2
TOTAL	15	11	14	13	11
%		73	93	87	73

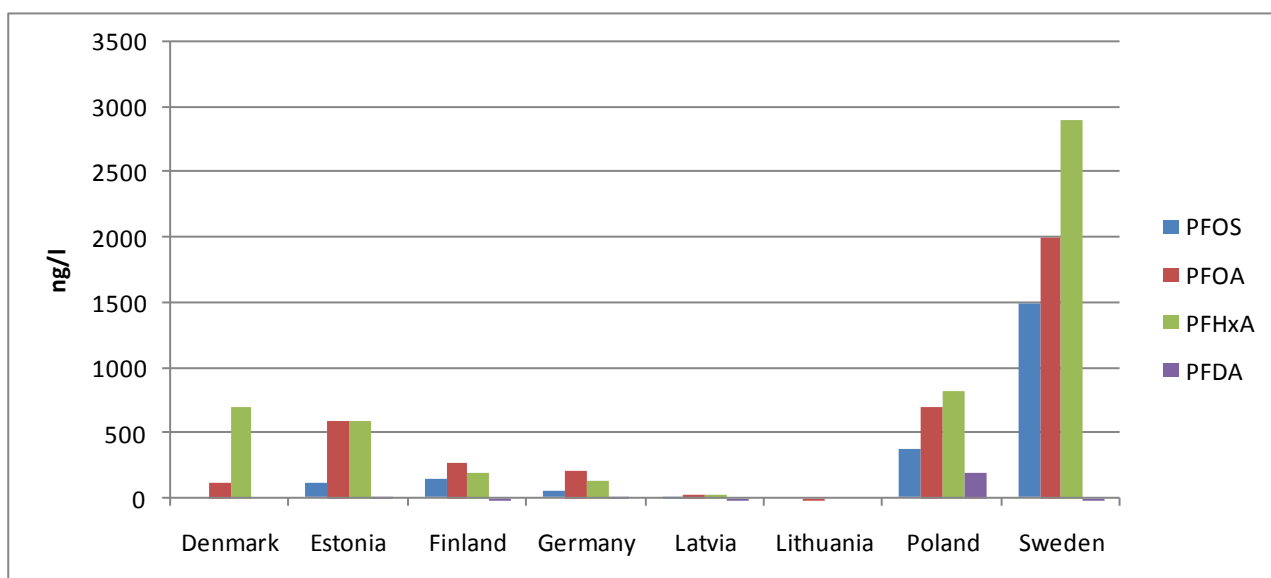


Figure 4.49. The highest concentrations (ng/l) of perfluoroalkyl acids found in landfill leachate samples.

In landfill leachates PFOA was the most frequently found perfluoroalkyl acid, but the highest concentrations were found for PFHxA. PFOA was found in every country and PFOS in all other countries except Lithuania.

Analytical method for sludge

In SYKE freeze-dried sample (1 g) was placed in a polypropene tube and surrogate standards ($^{13}\text{C}_2$ -PFHxA, $^{13}\text{C}_4$ -PFOS and $^{13}\text{C}_2$ -PFDA) were added. NaOH in methanol (200 mmol/l) was used for digestion. After 30 min, HCl in methanol (2 mol/l) was added for neutralisation. Analytes were extracted in wrist-action shaker twice with methanol. The extracts were concentrated, and then puri-

fied with activated carbon and glacial acetic acid. Purified extract was diluted with de-ionised water and $^{13}\text{C}_4$ -PFOA was added as a quantification standard. Analyses were performed with UPLC-TQ MS. The LC effluents were water and methanol buffered with ammonium acetate. Calibration was done using linear-only standards and the results were reported as a sum of both linear and branched isomers. The final results are recovery corrected and the blank samples were determined in the all sample series.

Sludge

LOQs varied between laboratories and substances (Table 4.70.). When LOQ had changed during the project, it is expressed as a range.

Table 4.70. LOQs ($\mu\text{g}/\text{kg}$) of perfluoroalkyl acids in sludge (n.i. not informed).

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	*				0.5			n.i.
PFOA	2.0 - 2.1				0.5			n.i.
PFHxA	2.0 - 2.4				0.5			n.i.
PFDA	*				0.5			n.i.

Altogether 22 sludge samples were analysed for perfluoroalkyl acids (Table 4.71.). PFOS and PFDA were observed in all sludge samples. PFOA and PFHxA were found in 82% and 55% of the samples, respectively. The highest concentration was found for PFOS in Finland (110 $\mu\text{g}/\text{kg}$), for PFOA in Sweden (5.2 $\mu\text{g}/\text{kg}$), for PFHxA in Poland (2.1 $\mu\text{g}/\text{kg}$) and for PFDA in Denmark (16 $\mu\text{g}/\text{kg}$) (Figure 4.50.).

Table 4.71. The number of samples and frequencies of perfluoroalkyl acid concentrations above LOQ in sludge.

	Number of samples	PFOS	PFOA	PFHxA	PFDA
Denmark	4	4	1	0	4
Estonia	3	3	3	1	3
Finland	2	2	2	2	2
Germany	3	3	3	3	3
Latvia	4	4	3	3	4
Lithuania	2	2	2	0	2
Poland	2	2	2	1	2
Sweden	2	2	2	2	2
TOTAL	22	22	18	12	22
%		100	82	55	100

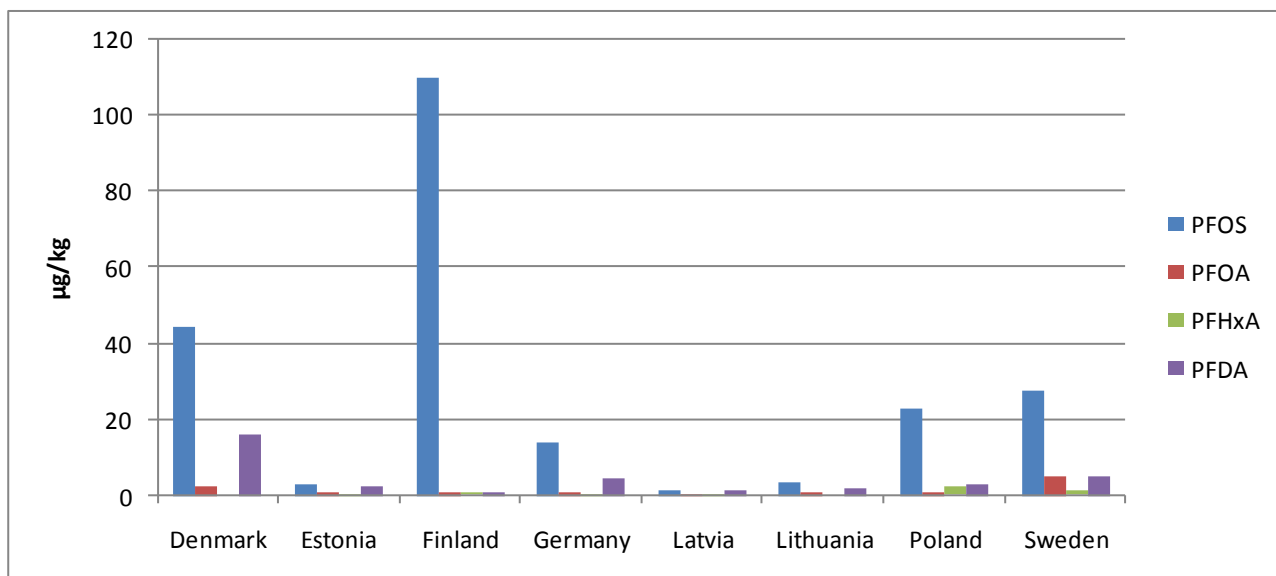


Figure 4.50. The highest concentrations ($\mu\text{g}/\text{kg}$) of perfluoroalkyl acids in sludge.

In sludge PFOS and PFDA was detected in all samples. The highest concentrations were found for PFOS. PFOS, PFOA and PFDA were found in every country.

4.9 Summary of chemical results

Most of the BSAP hazardous substances were found in every sample types included in the screening. In addition, bisphenol A, MBT, DBT, PFHxA and PFDA were found in each sample type. The detected frequencies are shown in table 4.72. It should be noted that the number of effluent samples was significantly higher than the number of sludge, landfill leachate and storm water samples. Many of the substances were found more often in sludge samples than in effluent samples. Findings in sludge indicate their presence in the effluents. Additional data from chemical analyses is presented in Appendix A.

For some substances, for example endosulfan and TBT, requirements for LOQs set by Commission Directive 2009/90/EC² can not be met by the methods used in this project.

² Commission Directive 2009/90/EC on Technical Specification for Chemical Analyses and Monitoring of Water Status.

Table 4.72. Summary of detection frequencies (concentration above LOQ) of substances and groups of substances. The differences in LOQs between laboratories have influenced the detection frequency.

Substance / substance group	Detection frequency (%)				
	municipal effluent	industrial effluent	storm water	landfill leachate	sludge
Cadmium, Cd	6	26	67	38	83
Mercury, Hg	68	46	73	62	100
MBT	59	50	33	60	79
DBT	50	39	40	26	79
TBT	7	2	27	20	79
TPhT	1	0	0	0	26
MOT	13	13	13	33	79
DOT	5	2	13	13	79
TCyT	0	0	0	0	26
Nonylphenols, NP	73	78	67	67	100
Nonylphenol ethoxylates, NPE	39	36	40	40	86
Octylphenols, OP	45	36	20	47	64
Octylphenol ethoxylates, OPE	17	16	27	33	36
Bisphenol A, BPA	58	45	93	100	27
Endosulfan	20	10	14	0	53
Endosulfan sulphate	24	20	14	29	33
Dioxins and furans , PCDD/F	74	65	89	75	69
Dioxin-like PCB, co-PCB	72	61	89	88	69
SCCP	92	91	93	93	100
MCCP	93	98	80	62	50
pentaBDE	26	21	60	67	91
octaBDE	2	6	20	40	91
decaBDE	65	68	67	67	91
HBCD	52	44	53	21	85
PFOS	86	62	87	73	100
PFOA	98	95	87	93	82
PFHxA	76	58	53	87	100
PFDA	58	15	40	73	55

5 Discussion of chemical results

There were differences in the waste water treatment processes in different countries. Influent depend on local conditions of the urban area e.g. pattern of indirect dischargers (industrial sites), use pattern of different kind of products, user behaviour, and regarding combined sewer system also pollutant load from urban runoff (roofs, streets etc.). Therefore, predicting the load of possible pollutants at MWWTPs has high uncertainty.

In industrial WWTP the effluent quality depends strongly on the branch of an industry. In the COHIBA project there were different kinds of industrial branches represented from oil refineries, pharmaceutical industry, ship yards and metal plating industries to coal power plants.

There were different treatment processes among the industrial or municipal treatment plants. The differences were revealed by the basic data parameters. The variation in the basic parameters does not, however, coincide with the presence of hazardous substances or biological effects. In general the detection frequencies all the 11 hazardous substances were quite similar between the municipal and industrial effluents.

There were many deviations from sampling programs agreed between partners during sampling campaigns (grab vs. composite, municipal vs. industrial), handling of samples (storage time and temperature, pre-treatment), number of samples taken and differences in analyses. For example in Latvia and Denmark there were only two municipal and industrial effluent samples for chemical analyses. There were also differences in the storm water and landfill leachate treatment, because in some countries they were treated and in others not. These should be kept in mind when comparing the results.

Substance specific discussion

Cadmium was detected from storm waters in all other countries, except Lithuania. The significantly high concentration was found from Poland. This may refer to high atmospheric deposition of cadmium due to coal use and industry. In municipal effluents Cd was generally detected in low levels. The high Cd concentration in the industrial effluents was observed in Germany, where samples were taken from coal power plant.

For mercury the exceeding of MAC-EQS value (0.07 µg/l) was remarkably in Danish and Polish municipal effluents. In industrial effluents the MAC – EQS value exceeding was observed in Germany, due to specific cooling system and process water, and in some degree in Latvia and Finland.

The concentrations of mercury were higher than LOQ in all sludge samples. According to Council Directive 86/278/EEC the maximum allowable concentration of mercury in sewage sludge can not be higher than 16 mg/kg dw. Mercury concentration in all sludge samples was below this. But the national legislations or restrictions can be even stricter. It seems that mercury in different type of waste waters discharged to Baltic Sea is still a problem in some countries.

The most frequently observed organotin compounds were mono- and di-butyltin (MTB and DBT). The concentrations in the municipal effluents and storm waters were about on the same level. In Denmark no tin compounds were observed in sludge samples and in Lithuania any in storm water samples.

The highest concentrations of the phenolic compounds were found in Swedish and Latvian storm water samples. Although in municipal effluent the concentrations were usually under 2.2 µg/l, the municipal sludge in Estonia and Poland contained, however, quite high concentration of phenolic substances. NPs are usually attached to solid substances and therefore remain in sludge. One additional reason for high NP concentrations in sludge might be transformation of NPE to NP in anaerobic conditions.

Endosulfans were observed in industrial and municipal effluents only in Finland, Germany, Latvia and Poland. In these countries, except in Latvia, endosulfans were found also in sludge. In storm waters endosulfans were found only in Finland and Germany. In landfill leachates only endosulfan sulphate was observed in Estonia, Germany and Finland. It should be noted that endosulfan is banned in the EU level. The German results were in contradiction with their earlier findings (from 2007) when out of 120 effluent samples studied from 20 MWWTPs, only in one case the detection limit of 0.007 µg/l was surpassed.

Highest TEQ values of the dioxin compounds were found in Germany, in all sample types. The highest amount in landfill leachates were observed in Poland and Finland (leachate data is not available from Germany).

In industrial and municipal effluents the concentrations of MCCPs and SCCPs were about at the same level, except in Denmark, where MCCP was detected at a very low concentration in municipal effluents. In the effluents MCCPs were prevailing while in the sludge SCCPs were more abundant. The only exception was Denmark where MCCP prevailed also in sludge. MCCPs and SCCPs were also detected in the storm water samples of all the participating countries.

The prevailing PBDE congener in almost all sample types was decaBDE. The highest concentration of decaBDEs in municipal effluents and storm waters was observed in Denmark. In industrial effluents the concentration of penta-, octa- and decaBDEs were highest in Lithuania. In landfill leachates also pentaBDE was detected quite frequently.

HBCDs were analysed in Denmark and Sweden as sum parameter, in all other countries the results were analysed as isomer concentrations (α -, β - and γ -HBCD). Therefore the results were not comparable. The prevailing isomer in municipal and industrial effluents was the γ -isomer, in sludge, landfill leachate and storm water the isomer distribution was more variable.

The highest concentrations of perfluorinated compounds were found in Finnish WWTP effluents. In municipal effluents the most probable source was the influent from a metal plating industry, and in industrial effluent the use of flame retardant and fire fighting foams. The other sources were not identified as clearly e.g. the high concentrations in Danish storm waters and Swedish landfill leachates.

6 Biological tests and methods

6.1 Obligatory tests

It was determined in the project application that *Vibrio fischeri* luminescent bacteria test, *Daphnia magna* acute toxicity test and algae growth inhibition test would be the obligatory test, which all participating parties should arrange either by performing or by purchasing as service. Laboratories used their own validated test methods. Finland performed all these obligatory tests for Denmark, and *Vibrio fischeri* luminescent bacteria tests for Estonia, while the Swedish samples were not tested for acute toxicity. The obligatory tests were performed according to standardised test methods (ISO standards: ISO 11348-3, luminescent bacteria tests; ISO 6341, *Daphnia magna* immobilisation tests; ISO 8692 algae growth inhibition test).

3,5-dichlorophenol (DCP) and potassium dichromate were used as reference chemicals. Samples and reference chemicals were analysed at several concentrations allowing the estimation of EC50-values, when the observed effect was strong enough. EC50-value is the concentration which causes 50 % effect of respective parameter of the exposed organisms within the standard test time. The detailed test methods and procedures are described in partners' WP3 National reports (www.cohiba-project.net).

Vibrio fischeri, luminescent bacteria test is one of the most common toxicity test used for waste water toxicity assessments. This method is suitable for many kinds of aqueous samples; surface and ground water, waste water, water extracts and leachates etc. Instead of the luminescent bacteria test, Latvia performed *Artemia salina* (brine shrimp) mortality test by using commercial ArtoxKit method.

The *Daphnia magna* standard describes a method for the determination of immobilisation of the animals during 24 or 48 hours.

Algae growth inhibition test specifies a method for the determination of the growth inhibition of unicellular green algae by substances and mixtures in water or wastewater. The growth inhibition test can be performed either in small volume cells, microplates, or in Erlenmeyer flasks.

6.2 Optional tests

The participants were encouraged to use also optional tests for the detection of possible hazardous effects of the effluents. Finnish and Lithuanian samples were tested with a number of optional tests (see results in Table 7.1.). In addition, all countries sent two of their effluent samples to Finland for testing with five optional tests (see results in Table 7.1.). More information of these test methods is available in the WP3 National reports (www.cohiba-project.net).

7 Results of biotests

The short-term toxicity tests showed occasionally acute toxicity in the effluents. Results are shown in table 7.1, where the incidence of positive results is presented as percentages of the total number of samples analysed. In table 7.2 results of municipal, industrial landfill leachate and storm water samples are sorted by country. In general, the results of the biotests showed that the quality of the effluents varied greatly from sampling time to sampling time. This was also observed in relation to the basic parameters of the effluents. An example of the variation in estrogenicity is shown in figure 7.1. Obviously, the toxicity testing should be repeated more frequently to confirm the results for individual plants.

Based on the results of the acute toxicity tests, the algae growth inhibition test seemed to be the most sensitive for both municipal and industrial effluents, and also for the storm waters. In many cases, at low concentrations in particular, there was stimulation of the algae growth. This growth stimulation is a common observation concerning effluents, and it might overpower the effects of toxins.

Both optional tests with aquatic crustaceans *Artemia salina* and *Thamnocephalus platyurus* were more sensitive to tested effluents than was *Daphnia magna* immobilization test (national reports of Latvia and Lithuania). Low toxicity can be identified with protozoa and rotifers kit tests. Relatively high detection and quantification potential has been demonstrated with freshwater macrophytic algae tests (national report of Lithuania). In spite of limited number of effluents tested, the data obtained throughout the project suggest that optional tests can also be useful for effluent toxicity control in distinct countries.

Aquatic animals are sensitive to exogenous chemical substances during reproduction. Also in this study, effects on organisms' reproduction were observed in the long-term water flea test and zebra fish egg-larvae test. *In vitro* tests with fish hepatocytes exhibited estrogenic and disturbed detoxification metabolism of xenobiotics by the majority of the effluent samples. These effects were most obvious in the municipal wastewater effluents (Table 7.1. and 7.2.). Estrogenicity of effluents is perhaps one reason for the results observed in *Daphnia magna* reproduction tests; exposed Daphnids produced significantly more eggs than the control animals.

Original effluents did not show genotoxicity when assayed by umu-test, but after sample concentration to 30 fold there where indication of genotoxicity. Ames test, however, showed genotoxicity even without concentration (national report of Poland). These tests measure different mechanisms of genotoxicity. Therefore, the observed effects are not directly comparable between effluents. The positive results in umu-test after concentration of the samples are an indication of the risk that these samples contain compounds, which by bioaccumulation may have adverse effects on aquatic organisms.

All toxicity test results are presented in more detail in partners' WP3 National reports.

Table 7.1. Toxicity incidence of positive results presented as percentages of the total number of effluent samples analysed. Green, no effects; yellow, toxicity below the recommended limit, red, above the recommended limit value (Appendix B page 115); n, the number of samples per test.

Obligatory tests	MWWTP				IWWTP			
	%	%	%	n	%	%	%	n
<i>Daphnia magna</i> acute	86	13	1	96	78	12	10	71
Algae growth inhibition	82	2	16	91	61	18	21	66
Luminescent bacteria test	95	4	1	74	92	8		50
Optional tests								
<i>Artemia salina</i>	31	63	6	8	19	75	6	8
<i>T. platyurus</i>	63	6	31	16	100			16
<i>N. obtusa</i> (Charatox)	38	31	31	16	44	50	6	16
<i>N. obtusa</i> (Niteltox)	63	6	31	16	100			16
<i>B. calyciflorus</i>	94	6		16	100			16
<i>T. thermophila</i>	93		7	14	86		14	14
Genotoxicity, umu-test*	82	18		28	80	20		11
Egg-larvae test	96	4		28	70	10	20	11
<i>D. magna</i> reproduction	100			28	100			11
<i>Lemna minor</i>	58	42		28	60	40		11
Vitellogenin test			100	28			100	11
Hepatocyte EROD activity			100	28			100	11

Obligatory tests	Landfill leachates				Stormwaters			
	%	%	%	n	%	%	%	n
<i>Daphnia magna</i> acute	33		67	12	75	8	17	12
Algae growth inhibition	25	8	67	12	64	9	27	11
Luminescent bacteria test	20		80	10	100			10
Optional tests								
<i>Artemia salina</i>	100			1	100			1
<i>T. platyurus</i>		50	50	2	100			2
<i>N. obtusa</i> (Charatox)	100			2	50	50		2
<i>N. obtusa</i> (Niteltox)	50		50	2	100			2
<i>B. calyciflorus</i>	100			2	100			2
<i>T. thermophila</i>	100			2	100			2
Genotoxicity (umu-test*)	100			2	100			2
Egg-larvae test			100	2	100			2
<i>D. magna</i> reproduction	50		50	2	100			2
<i>Lemna minor</i>	50	50		2	50	50		2
Vitellogenin test			100	2			100	2
Hepatocyte EROD activity			100	2			100	2

* in umu-tests the yellow values are from concentrated samples

Table 7.2. Number of municipal (MWWTP) and industrial (IWWTP) effluents, landfill leachates (LL) and storm waters (STW) samples sorted by country colour coded by toxicity. Green, no effects; yellow, toxicity below the recommended limit (Appendix B page 115); red, above the recommended limit value; white, no samples tested.

Municipal effluents																													
Obligatory tests		DK			EE			FI			DE			LV			LT			PL			SE						
<i>Daphnia magna</i> , acute		4			10	1	1	16	2		14			14	2		14			11	7								
Algae growth inhibition		1	1	2	8	1	3	13	1	4	14			11			10		4	18									
Luminescent bacteria test		4			10	2		18			14						8			16	1	1							
Optional tests																													
Genotoxicity, umu-test*		1	1					16	2			2			2			1			15	2	1	2					
Egg-larvae-test		2						18			1	1		2			1			1			2						
<i>D. magna</i> , reproduction								18			2																		
<i>Lemna minor</i>		1	1					11	5					2			1			1			1	1					
Vitellogenin test				2						18			2			2			1			1						2	
Hepatocyte EROD activity				2						18			2			2			1			1						2	

Industrial effluents																													
Obligatory tests		DK			EE			FI			DE			LV			LT			PL			SE						
<i>Daphnia magna</i> , acute		4			11			5	1		11	1	2	9	5	2	14			2	1	3							
Algae growth inhibition				4	5	5		3		3	11	2	1	4	5	3	11		3	6									
Luminescent bacteria test		2	2		11	1		5	1		14						8			6									
Optional tests																													
Genotoxicity, umu-test*					2			4	2								1			6									
Egg-larvae-test						1	1	5		1							1			1									
<i>D. magna</i> , reproduction								18																					
<i>Lemna minor</i>					1	1		4	2								1				1								
Vitellogenin test							2			6								1				1							
Hepatocyte EROD activity							2			6									1			1							

Obligatory tests	Denmark				Estonia				Finland				Germany																
	LL		STW		LL		STW		LL		STW		LL		STW														
<i>Daphnia magna</i> , acute			2	1			2	2		1		1	2		1					1	1								
Algae growth inhibition			2			1			1	1	1	1			2	1		1	1								2		
Luminescent bacteria test			2	1				2	2					2	2					1							2		
Optional tests																													
Genotoxicity, umu-test*													2				2												
Egg-larvae-test															2	1	1												
<i>D. magna</i> , reproduction													1		1	2													
<i>Lemna minor</i>														1	1	1	1												
Vitellogenin test																2													
Hepatocyte EROD activity																2													

Obligatory tests	Latvia		Lithuania		Poland		Sweden										
	LL	STW	LL	STW	LL	STW	LL	STW									
<i>Daphnia magna</i> , acute		1	1		2		2			2		2					
Algae growth inhibition					2		2			2	1		1				
Luminescent bacteria test					1		1			2	2						

* in umu-tests the yellow values are from concentrated samples

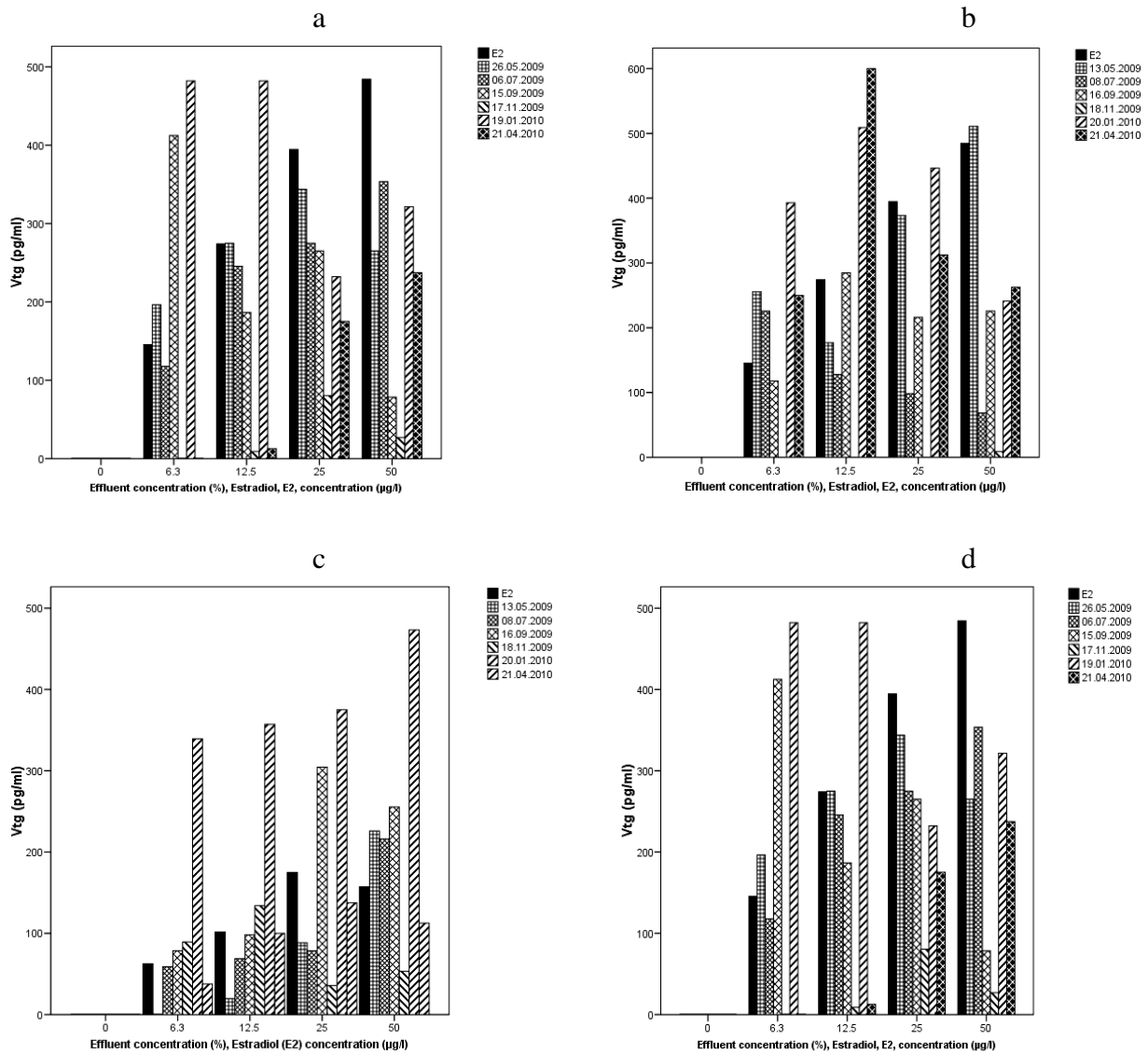


Figure 7.1. An example of vitellogenin induction results in male fish liver cells exposed to one Finnish municipal (a-c) and industrial (d) effluent to demonstrate the variation in effluent quality. E2, 17 β -estradiol was the positive control.

8 Discussion of biotest results

Harmonization of methods

One of the COHIBA project's aims was to harmonise both the chemical and ecotoxicological methods in the Baltic Sea region in order to have comparable and reliable results for assessments and evaluation of sources for hazardous substance. For this purpose the comparison tests (ring-tests) were organised within the project for verifying the congruence between results of different laboratories. The other target for the ring-test was to evaluate the performance of analytical methods and find out which of the methods can be recommended for monitoring (www.cohiba-project.net).

In toxicity testing there are several sources of uncertainty; differences in the sensitivity of test organisms, different laboratory practices, test conditions and procedures etc. Also these factors should be taken into account when harmonizing methods or elaborating country specific recommendations and practices.

Finnish Environment Institute (SYKE) organised the ring-tests for "obligatory" biotests. Tests were performed according ISO standards. Each ISO standard gives the criteria of validity for the results. One requirement is that the EC50-value of the reference compounds given in the standard, lies between the given range values.

Biological effects

COHIBA WP3 has characterised the effluents from municipal and industrial waste water plants landfill leachates, storm waters, which were to represent the general quality of discharges to the Baltic Sea. The sampling campaigns were to represent concurrent discharges from different countries. The analyses and tests carried out by the project have built substantially the knowledge on these issues. Comparable results for such a large geographic area can be gained only by close co-operation between partners and using well-established methods and procedures. Co-operation of wastewater treatment plants, other stakeholders and analysing laboratories is a prerequisite of success in gaining representative samples and reliable results.

In this project conventional short-term toxicity tests, *Daphnia magna* immobilization, luminescent bacteria and algae growth inhibition test, were regarded as obligatory. There are plenty of available data to show that algae and daphnias are ecologically important species. Luminescent bacteria test has been shown to correlate well with the other aquatic tests such as fish and daphnia tests. These three methods are standardised and are widely accepted as suitable for waste water effluent testing. Nevertheless, some small differences between laboratories are possible during the execution of the tests. Concerning both biological and chemical methods, it is important that the congruence of the results is examined regularly, and that best practices are adopted in all laboratories producing data for permission compliance or for international use e.g. for HELCOM monitoring.

In general biotests should be conducted with several (preferably 3-5) different organisms on different trophic levels/functional groups (e.g., bacteria, phytoplankton, invertebrates and fish). There are several reasons for this. Firstly, there is variation between the sensitivity of the different species, and therefore using several species increases the reliability of the tests. Secondly, no single species can indicate all the substantial end-points. This is a way to obtain a better view of the effects on ecosystem functioning and on different trophic levels.

One of the advantages of the WEA approach, concerning long-term tests in particular, is that it provides a more comprehensive picture of the effluent's environmental effects than the chemical concentration data. WEA can be seen as a "link between chemistry and ecology" as it indicates directly the effects of an effluent on survival, growth and/or reproduction of organisms. The chemical analysis based on extracting and determining concentrations of individual chemicals does not adequately simulate the situation where living cells/organisms are exposed to the whole effluent. All the substances present in the effluent affect the metabolism of test organisms to some extent. This is taken into account in WEA, which detect the effects of unknown substances and synergistic effects. Many important biological processes (such as bioavailability and bioaccumulation, selective intake in a cell, xenobiotic metabolism etc.) are an inherent part of WEA tests, since living cells/organisms are used.

Long-term toxicity tests are not as straight-forward to perform as the acute tests. They need more training and experience, and they are more laborious. Selection of the appropriate long-term toxicity test methods and organisms may also need revision from time to time. The methods should be capable to detect toxicity after long-term exposure, effects on important biological functions such as early life development, reproduction, growth, detoxification, bioaccumulation and hormonal functions. There are several international standard protocols for the long-term tests, which are suitable for wastewater testing.

Effluents containing small concentrations of certain bioaccumulating substances such as brominated flame retardants can have an adverse impact on the Baltic Sea. Some of the priority substances for example perfluorinated compounds and bisphenol A, are so persistent that their concentrations will inevitable increase and result in constant exposure of the marine biota.

Various chemicals are released from consumer products and many other sources. Despite restrictions of the most hazardous ones within EU, they still exist in wastewater effluents. Therefore, it is important to raise the public awareness of the possible environmental risks of the chemicals, and to adjust control and monitoring programmes adequately. Also purification processes should be optimized to achieve a more constant effluent quality (e.g. source control, adequate treatment capacity regarding increased population).

9 Conclusions

Thorough planning for sampling and organizing the practical arrangements along with a complete documentation are the basis for good-quality samples and results concerning both chemical and biological analyses. Considering the final interpretation of the results the proper documentation of the sampling methods (grab vs. composite sample, sample containers, pre-treatment of samples (*e.g.* filtrations, preservation), and storage (time, conditions, temperature etc.) are essential information. The representativeness of the samples is the key to further appropriate actions.

High quality analytical methods are valuable in controlling individual substances and identifying substances of restricted use or banned substances. Analytical performance, well established quality control procedures and professionally skilled personnel are highly valued in the laboratory work. Applied methods shall be properly validated and performance should be known and documented (*e.g.* sensitivity, selectivity and accuracy). Methods sensitivities should meet the legislative and permission requirements³. Regarding both chemical and toxicity test methods; methods development and testing of applicability to effluent control should be continued.

For a number of substances within the hazardous BSAP substances, COHIBA project was one of the first occasions to estimate the discharges to and the concentrations in the Eastern Baltic Sea region. The screening gave new information on the presence of hazardous substances in the Eastern Baltic Sea area and those results have changed the prevalent opinion that there are no problems with hazardous substances in that area. It is also clear that there is a great need for additional measures for treating wastewaters. On the basis of COHIBA results, it is possible to plan future studies, reduction measures and national monitoring for those selected substances.

WEA offers a practical and flexible tool for assessing the effluent quality in an effective way. It enables the assessment of potential risks and effects for both identified and unidentified substances. By using WEA in combination with chemical analyses it is possible to identify sources of hazardous substances and to plan preventive actions. This procedure should be an effective tool to increase the level of protection of the Baltic Sea and enhance its ecological status.

³ COHIBA Recommendation report www.cohiba-project.net

Appendix A

Cadmium

Maximum concentrations ($\mu\text{g/l}$) of cadmium found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Cadmium	<0.05	0.14	0.07	0.12	0.2	<0.05	0.75	<0.2

Maximum concentrations ($\mu\text{g/l}$) of cadmium found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Cadmium	0.83	0.15	<0.01 / <0.05	4.0	0.3	<0.05	0.40	-

Maximum concentrations ($\mu\text{g/l}$) of cadmium found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Cadmium	0.13	0.16	0.06	0.27	0.9	<0.05	18.1	0.28

Maximum concentrations ($\mu\text{g/l}$) of cadmium found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Cadmium	<0.05	<0.02	0.29	0.087	0.1	<0.05	1.46	0.39

Maximum concentrations (mg/kg) of cadmium found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Cadmium	1.5	<1	0.69	0.64	-	0.52	3.44	0.8

Mercury

Maximum concentrations ($\mu\text{g/l}$) of mercury found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Mercury	0.94	0.05	0.027	<0.1*	-	0.029	0.054	0.81

Maximum concentrations ($\mu\text{g/l}$) of mercury found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Mercury	<0.05	0.05	0.17	4.4	0.32	0.044	0.063	-

Maximum concentrations ($\mu\text{g/l}$) of mercury found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Mercury	0.29	<0.05	0.012	0.0054	<0.1	0.023	0.21	0.042

Maximum concentrations ($\mu\text{g/l}$) of mercury found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Mercury	0.13	0.1	0.037	0.140	0.38	<0.020	0.47	0.041

Maximum concentrations (mg/kg) of mercury found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Mercury	2.7	0.5	1	1.10	-	0.25	1.5	0.81

Organotins

Maximum concentrations (ng/l) of organotin compounds found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
MBT	12	8.9	17	15.0	4.2	18	10	6.4
DBT	<5	7.5	9.6	17.0	1.3	1.3	5	6.8
TBT	<4	2.9	1.1	1.3	<1.0	<1	2.7	<1
TTBT	-	5.5	<1.0	<1	<1.0	<1	3.2	1.2
MOT	-	3.7	<1.0	1.5	<1.0	<1	9.4	1.6
DOT	-	1.3	0.99	<1	<1.0	<1	1.3	<1
TPhT	<1	<1	<1.0	<1	<1.0	<1	1.07	<1
TCyT	-	<1	<1.0	<1	<1.0	<1	<1	<1

Maximum concentrations (ng/l) of organotin compounds found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
MBT	16	10	2.7	23	2.2	2.4	1.9	-
DBT	34	5.6	9.2	32	1.7	1.4	4.75	-
TBT	110	<1	<1.0	<1	<1.0	<1	<1	-
TTBT	-	13	<1.0	<1	<1.0	<1	110	-
MOT	-	8.5	<1.0	4.1	<1.0	<1	96	-
DOT	-	<1	<1.0	<1	<1.0	<1	4.9	-
TPhT	<1	<1	<1.0	<1	<1.0	<1	<1	-
TCyT	-	<1	<1.0	<1	<1.0	<1	<1	-

Maximum concentrations (ng/l) of organotin compounds found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
MBT	18	<1	3	2.0	<1	<1	<1	20
DBT	10	5.4	22	2.6	4	<1	<1	9
TBT	<4	<1	<1.0	<1	5	<1	1.2	<1
TTBT	-	<1	<1.0	<1	<1	<1	<1	
MOT	-	1.5	<1.0	<1	<1	<1	<1	11
DOT	-	<1	<1.0	<1	1.1	<1	<1	1.6
TPhT	<1	<1	<1.0	<1	<1	<1	<1	<1
TCyT	-	<1	<1.0	<1	<1	<1	<1	<1

Maximum concentrations (ng/l) of organotin compounds found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
MBT	12	58	84	<1	19	59	780	45
DBT	<25	<5	12	1.3	<5	<5	60	<5
TBT	<20	<5	12	<1	<5	<5	280	<5
TTBT	-	<5	<1.0	<1	<5	<5	43	<5
MOT	-	18	33	<1	<10	9.3	100	<10
DOT	-	<10	<1.0	<1	<10	<10	31	<10
TPhT	<5	<5	<1.0	<1	<5	<5	<1	<5
TCyT	-	<5	<1.0	<1	<5	<5		<5

Maximum concentrations ($\mu\text{g}/\text{kg}$) of organotin compounds found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
MBT	<2	420	740	620	600	560	480	530
DBT	<20	230	600	390	370	240	280	380
TBT	<7	4.1	9.1	16	53	4.6	120	14
TTBT	-	nd	<LOQ	-	-	*		-
MOT	-	83	200	420	200	180	320	120
DOT	-	33	250	410	110	85	210	59
TPhT	<3	<5	<LOQ	<5	9.1	<5	7	<5
TCyT	-	nd	<LOQ	<1	8.7	1.2		<5

Phenolic substances

Limit of quantifications varies between laboratories and phenolic substances. In case where LOQ had changed during the project the LOQ is expressed as a range.

LOQs ($\mu\text{g/l}$) for phenolic compounds in municipal effluent. If all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and LOQ is given in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	0.1			0.10 (0.35)				0.01
NP	0.1			0.10 (0.35)				0.05
NP1EO	0.05 - 0.1			0.05 (0.17)				0.05
NP2EO	0.1			0.05 (0.07)				0.05
OP	0.1			0.05 (0.17)				0.01
OP1EO	0.1			0.02 (0.07)				0.01
OP2EO	0.1			0.02 (0.07)				0.01

Number of samples and observed frequencies of phenolic compounds in municipal effluents. In case where detection limit were used as limit for observations, the number of observation above LOQ given in parenthesis.

	Number of samples	BPA	4-NP	4-NP1EO	4-NP2EO	OP	OP1EO	OP2EO
Denmark	6	3	3	0	0	0	0	0
Estonia	12	9 (5)	10 (3)	8 (0)	9 (2)	7 (2)	2 (0)	0
Finland	18	15 (6)	17 (9)	9 (0)	14 (3)	7 (1)	2 (0)	0
Germany	12	4 (0)	12 (3)	7 (0)	0	5 (3)	2 (0)	0
Latvia	4	2 (0)	4	3 (0)	3 (0)	2	0	0
Lithuania	12	7 (2)	11 (3)	7 (0)	4 (1)	2 (0)	0	0
Poland	18	15 (5)	18 (11)	14 (4)	13 (5)	10 (5)	2 (0)	0
Sweden	24	16	9	4	2	24	13	5
TOTAL	106	71	84	52	45	57	21	5
%		67	79	49	42	54	20	5

Maximum concentrations ($\mu\text{g/l}$) of phenolic compounds found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	0.93	5.8	0.72	0.17	0.21	0.39	1.1	1.9
4-NP	0.32	0.54	1.2	2.2	0.66	0.75	1.3	0.11
4-NP1EO	<0.1	0.1	0.12	0.07	0.08	0.11	0.5	0.11
4-NP2EO	<0.1	0.19	0.10	<0.02	0.03	0.09	0.11	0.07
OP	<0.1	0.26	0.24	0.26	0.26	0.13	0.32	0.31
OP1EO	<0.1	0.02	0.02	0.04	<0.02	<0.02	0.02	0.51
OP2EO	<0.1	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.24

LOQ ($\mu\text{g/l}$) for phenolic compounds in industrial effluent. If all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and LOQ is given in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	0.02-0.1			0.10 (0.35)				-
NP	0.05-0.1			0.10 (0.35)				-
NP1EO	0.05-0.1			0.05 (0.17)				-
NP2EO	0.1			0.05 (0.07)				-
OP	0.1			0.05 (0.17)				-
OP1EO	0.1			0.02 (0.07)				-
OP2EO	0.1			0.02 (0.07)				-

Number of samples and found frequencies of phenolic compounds in industrial effluents. When detection limit s were used as limit for observations, the number of observation above LOQ given in parenthesis.

	Number of samples	BPA	4-NP	4-NP1EO	4-NP2EO	OP	OP1EO	OP2EO
Denmark	4	2	1	1	1	0	1	1
Estonia	12	8 (2)	12 (8)	10 (6)	10 (8)	8 (2)	7 (4)	5 (4)
Finland	6	5 (2)	4 (2)	2 (0)	3 (0)	2 (0)	1 (0)	0
Germany	11	1 (0)	10 (8)	3 (0)	0	6 (2)	0	0
Latvia	4	2 (0)	4 (0)	1 (0)	0	2	0	0
Lithuania	12	5 (0)	7 (2)	3 (0)	0	1	0	0
Poland	6	4 (3)	5	2 (0)	2 (1)	2 (1)	1 (0)	0
Sweden	0	-	-	-	-	-	-	-
TOTAL	55	27	43	22	16	21	10	6
%		49	78	40	29	38	18	11

Maximum concentrations ($\mu\text{g/l}$) of phenolic compounds found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	92	1.6	0.91	0.28	0.13	0.3	1.4	-
4-NP	0.14	2.6	0.70	2.1	0.32	0.5	0.93	-
4-NP1EO	0.33	6.4	0.1	0.05	0.05	0.06	0.07	-
4-NP2EO	0.29	7.0	0.04	<0.02	<0.02	<0.02	0.31	-
OP	<0.1	0.22	0.09	0.26	0.36	0.19	0.02	-
OP1EO	3.8	1.3	0.02	<0.02	<0.02	<0.02	<0.02	-
OP2EO	25	3.6	<0.02	<0.02	<0.02	<0.02	<0.02	-

LOQ ($\mu\text{g/l}$) for phenolic compounds in storm water. In case all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and LOQ is given in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	0.1			0.10 (0.35)				0.1
NP	0.1			0.10 (0.35)				0.1
NP1EO	0.05 - 0.1			0.05 (0.17)				0.7
NP2EO	0.1			0.05 (0.07)				0.7
OP	0.1			0.05 (0.17)				0.04
OP1EO	0.1			0.02 (0.07)				0.05
OP2EO	0.1			0.02 (0.07)				0.05

Number of samples and noted frequencies of phenolic compounds in storm water. In case where detection limit were used as limit for observations, the number of observation above LOQ given in parenthesis.

	Number of samples	BPA	4-NP	4-NP1EO	4-NP2EO	OP	OP1EO	OP2EO
Denmark	2	2	2	0	0	0	0	0
Estonia	2	2 (0)	1 (0)	2 (0)	2 (1)	0	1 (0)	0
Finland	2	2 (1)	2 (1)	2 (0)	1	0	0	0
Germany	2	2	1 (0)	0	1	0	1 (0)	0
Latvia	1	1	1	1	1	1	1 (0)	0
Lithuania	2	2 (0)	0	0	0	0	0	0
Poland	2	1	2 (1)	1 (0)	1 (0)	1 (0)	0	0
Sweden	2	2	2	1	1	2	1	1
TOTAL	15	14	11	7	7	4	4	1
%		93	73	47	47	27	27	7

Maximum concentrations ($\mu\text{g/l}$) of phenolic compounds found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	0.32	0.24	0.62	3.1	0.73	0.14	0.37	0.42
4-NP	0.19	0.23	0.38	0.17	2.6	0.19	0.42	2
4-NP1EO	<0.1	0.08	0.12	<0.05	0.9	<0.05	0.07	4.7
4-NP2EO	<0.1	0.09	0.13	0.09	0.2	<0.02	0.06	0.18
OP	<0.1	<0.05	<0.05	<0.05	0.24	<0.05	0.14	0.11
OP1EO	<0.1	0.03	<0.02	0.02	0.02	<0.02	<0.02	0.24
OP2EO	<0.1	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.47

LOQ ($\mu\text{g/l}$) for phenolic compounds in landfill leachate. In case where all observations were recorded the detection limit ($\mu\text{g/l}$) is informed and LOQ is given in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	0.1	0.10 (0.35)						0.05
NP	0.05-0.1	0.10 (0.35)						0.05-0.15
NP1EO	0.05-0.1	0.05 (0.17)						0.05-0.15
NP2EO	0.1	0.05 (0.07)						0.05-0.2
OP	0.1	0.05 (0.17)						0.01-0.05
OP1EO	0.1	0.02 (0.07)						0.01-0.05
OP2EO	0.1	0.02 (0.07)						0.01-0.05

Frequencies of phenolic compounds observed in landfill leachate. When detection limits were used as limit for observations, the number of observation above LOQ is given in parenthesis.

	Number of samples	BPA	4-NP	4-NP1EO	4-NP2EO	OP	OP1EO	OP2EO
Denmark	2	2	0	0	0	0	0	0
Estonia	2	2	2	0	2 (1)	1 (0)	2 (0)	0
Finland	2	2	2	1	2	1	2 (1)	0
Germany	2	2 (1)	1 (0)	0	0	1	1	1 (0)
Latvia	1	1	0	0	1	1 (0)	0	0
Lithuania	2	2	1 (0)	0	0	0	0	0
Poland	2	2	2	2	1	1	1 (0)	1
Sweden	2	2	2	0	0	2	0	0
TOTAL	15	15	10	3	6	7	6	2
%		100	67	20	40	47	40	13

Maximum concentrations ($\mu\text{g/l}$) of phenolic compounds found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	0.97	0.65	300	0.43	2.7	7.3	700	0.08
4-NP	<0.1	0.99	1.7	0.10	<0.10	0.23	15	0.24
4-NP1EO	<0.1	<0.05	0.21	<0.05	<0.05	<0.05	2.0	<0.15
4-NP2EO	<0.1	0.09	0.08	<0.02	0.08	<0.02	0.2	<0.2
OP	<0.1	0.07	0.26	0.11	0.16	<0.05	1.0	0.08
OP1EO	<0.1	0.03	0.07	0.07	<0.02	<0.02	0.05	<0.05
OP2EO	<0.1	<0.02	<0.02	0.02	<0.02	<0.02	0.09	<0.05

LOQ (mg/kg) for phenolic compounds in sludge. In case where all observations were recorded the detection limit (mg/kg) is informed and LOQ is given in parenthesis.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	*			0.10 (0.35)				*
NP	*			0.10 (0.35)				*
NP1EO	0.6			0.05 (0.17)				*
NP2EO	0.6			0.05 (0.07)				*
OP	0.1			0.05 (0.17)				*
OP1EO	0.1			0.02 (0.07)				*
OP2EO	0.1			0.02 (0.07)				*

* not informed

Number of samples and noted frequencies of phenolic compounds in sludge. In case where detection limit was used as limit for observations, the number of observation above LOQ was put in parentheses.

	Number of samples	BPA	4-NP	4-NP1EO	4-NP2EO	OP	OP1EO	OP2EO
Denmark	4	4	4	2	0	0	1	1
Estonia	3	0	3	2	2	1	2 (1)	2 (1)
Finland	2	0	2	2	2 (1)	2 (1)	2 (1)	0
Germany	3	0	3	3	3	3 (0)	0	0
Latvia	4	0	4 (2)	4 (2)	4 (3)	3 (0)	0	0
Lithuania	2	0	2 (1)	2 (1)	2	2 (0)	0	1
Poland	2	0	2	2	2 (1)	2 (1)	2 (1)	1 (0)
Sweden	2	2	2	2	2	2	2	2
TOTAL	22	6	22	19	17	15	9	7
%		27	100	86	77	68	41	32

Maximum concentrations (mg/kg) of phenolic compounds found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
BPA	0.25	<0.30	<0.03	<0.30	<0.30	<0.30	<0.3	0.42
4-NP	8.6	24	12	3.0	15	4.3	37	9.7
4-NP1EO	0.7	31	3.4	2.6	1.7	0.72	8.4	4.4
4-NP2EO	<0.6	26	0.43	1.0	0.59	0.9	2.4	0.65
OP	<0.1	0.77	1.3	0.21	0.33	0.2	0.76	0.46
OP1EO	0.15	5.1	0.2	<0.05	<0.05	<0.05	0.29	0.10
OP2EO	4.1	9.6	<0.05	<0.05	<0.05	0.31	0.08	0.76

Endosulfans

LOQ ($\mu\text{g/l}$) for endosulfans in municipal effluent. If all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and the limit of quantification is given in parentheses.

	Denmark	Estonia	Sweden	Lithuania	Germany	Latvia	Finland	Poland
α -endosulfan	(0.01)	(0.005)	(0.001)	0.002 (0.004)	(0.01)			
β -endosulfan	(0.01)	(0.005)	(0.001)	0.002 (0.004)	(0.01)			
endosulfan sulphate	(0.01)	(0.005)	(0.001)	0.003 (0.005)	(0.01)			

Number of samples and observed frequencies of endosulfans in municipal effluents.

	Number of samples	α -endosulfan	β -endosulfan	endosulfan sulphate
Denmark	2	0	0	0
Estonia	12	0	0	0
Finland	18	3	3	4
Germany	12	5	2	5
Latvia	3	3	3	3
Lithuania	12	0	0	0
Poland	18	6	4	8
Sweden	24	3	3	4
TOTAL	101	20	15	24
%		20	15	24

Maximum concentrations ($\mu\text{g/l}$) of endosulfans found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -endosulfan	<0.01	<0.005	0.045	0.09	0.095	<0.004	0.079	<0.001
β -endosulfan	<0.01	<0.005	0.046	0.128	0.097	<0.004	0.031	<0.001
endosulfan sulphate	<0.01	<0.005	0.02	0.083	0.065	<0.004	0.13	<0.001

Number of taken samples and LOQ ($\mu\text{g/l}$) for endosulfans in industrial effluent. If all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and the limit of quantification is given in parentheses.

	Denmark	Estonia	Sweden	Lithuania	Germany	Latvia	Finland	Poland
α -endosulfan	(0.01)	(0.005)	(0.001)	0.002 (0.004)	(0.01)			
β -endosulfan	(0.01)	(0.005)	(0.001)	0.002 (0.004)	(0.01)			
endosulfan sulphate	(0.01)	(0.005)	(0.001)	0.003 (0.005)	(0.01)			

Number of samples and recorded frequencies of endosulfans in industrial effluents.

	Number of samples	α -endosulfan	β -endosulfan	endosulfan sulphate
Denmark	2	0	0	0
Estonia	12	0	0	0
Finland	6	0	0	1
Germany	11	3	2	6
Latvia	4	2	2	2
Lithuania	12	0	0	0
Poland	6	1	1	2
Sweden	0	0	0	0
TOTAL	53	6	5	11
%		11	9	21

Maximum concentrations ($\mu\text{g/l}$) of endosulfans found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -endosulfan	<0.01	<0.005	<0.01	0.051	0.047	<0.004	0.11	-
β -endosulfan	<0.01	<0.005	<0.01	0.2	0.066	<0.004	0.023	-
endosulfan sulphate	<0.01	<0.005	0.025	0.045	0.037	<0.005	0.04	-

LOQ ($\mu\text{g/l}$) for endosulfans in storm water. In case all observations were recorded, the detection limit ($\mu\text{g/l}$) is presented and the limit of quantification is given in parenthesis.

	Denmark	Estonia	Sweden	Lithuania	Germany	Latvia	Finland	Poland
α -endosulfan	(0.01)	(0.005)	(0.001)	0.002 (0.004)	(0.01)			
β -endosulfan	(0.01)	(0.005)	(0.001)	0.002 (0.004)	(0.01)			
endosulfan sulphate	(0.01)	(0.005)	(0.001)	0.003 (0.005)	(0.01)			

Number of samples and noted frequencies of endosulfans in storm water.

	Number of samples	α -endosulfan	β -endosulfan	endosulfan sulphate
Denmark	1	0	0	0
Estonia	2	0	0	0
Finland	2	1	0	1
Germany	2	1	1	1
Latvia	1	0	0	0
Lithuania	2	0	0	0
Poland	2	0	0	0
Sweden	2	0	0	0
TOTAL	14	2	1	2
%		14	7	14

Maximum concentrations ($\mu\text{g/l}$) of endosulfans found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -endosulfan	<0.01	<0.005	0.028	0.014	<0.01	<0.004	<0.01	<0.001
β -endosulfan	<0.01	<0.005	<0.01	0.032	<0.01	<0.004	<0.01	<0.001
endosulfan sulphate	<0.01	<0.005	0.01	0.016	<0.01	<0.005	<0.01	<0.001

LOQ ($\mu\text{g/l}$) for endosulfans in landfill leachate. When all observations were recorded the detection limit ($\mu\text{g/l}$) is informed and limit of quantification is in parenthesis.

	Denmark	Estonia	Sweden	Lithuania	Germany	Latvia	Finland	Poland
α -endosulfan	(0.01)	(0.005)	(0.001)	0.002 (0.004)	(0.01)			
β -endosulfan	(0.01)	(0.005)	(0.001)	0.002 (0.004)	(0.01)			
endosulfan sulphate	(0.01)	(0.005)	(0.001)	0.003 (0.005)	(0.01)			

Maximum concentrations ($\mu\text{g/l}$) of endosulfans found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -endosulfan	<0.01	<0.005	<0.01	<0.01	<0.01	<0.004	<0.01	<0.001
β -endosulfan	<0.01	<0.005	<0.01	<0.01	<0.01	<0.004	<0.01	<0.001
endosulfan sulphate	<0.01	0.038	0.16	0.01	<0.01	<0.005	<0.01	<0.001

Maximum concentrations (mg/kg d.w.) of endosulfans found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -endosulfan	<0.001	<0.001	0.86	0.58	-	0.0022	0.24	<0.001
β -endosulfan	<0.005	0.0013*	0.64	0.53	-	<0.001	0.26	<0.001
endosulfan sulphate	<0.001	<0.001	0.72	1.45	-	<0.001	0.064	<0.001

* - only in industrial sludge sample, in municipal sludge samples concentration of endosulfans were below the LOQ

Dioxins/furans, PCB

Maximum concentrations (pg/l) found in municipal effluents. Maximum values are expressed as WHO-TEQ 2005 upperbound.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	1.5	1.1	0.71	3.1	0.92	0.85	0.69	0.36
co-PCB	0.09	0.02	0.04	0.66	0.04	0.01	0.02	0.04

Maximum concentrations (pg/l) found in industrial effluents. Maximum values are expressed as WHO-TEQ 2005 upperbound.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	1.3	0.90	0.43	6.7	0.46	1.5	0.30	-
co-PCB	0.06	0.03	0.02	1.1	0.16	0.03	0.01	-

Maximum concentrations (pg/l) found in storm water. Maximum values are expressed as WHO-TEQ 2005 upperbound.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	1.36	1.63	0.46	10.8	2.3	0.96	1.23	5.40
co-PCB	0.19	0.05	0.04	17.5	0.88	0.02	0.02	4.60

Maximum concentrations (pg/l) found in landfill leachate. Maximum values are expressed as WHO-TEQ 2005 upperbound.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	0.55	1.5	4.8	-	0.45	0.78	13	0.62
co-PCB	0.04	0.25	0.96	-	0.16	0.01	2.3	0.17

Maximum concentrations (ng/kg) found in sludge. Maximum values are expressed as WHO-TEQ 2005 upperbound.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PCDD/F	5.3	-	3.8	770	15	3.1	11	
co-PCB	3.7	-	0.79	79	2.3	0.65	1.2	

Chlorinated paraffins

Maximum concentrations ($\mu\text{g/l}$) of chloroparaffins found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	0.04	1.98	2.09	1.71	1.2	1.95	2.67	2.13
MCCP	0.05	4.84	4.65	6.12	6.55	31.54	10	16.21

Maximum concentrations ($\mu\text{g/l}$) of chloroparaffins found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	0.038	2.94	2.48	1.89	3.61	1.45	3.25	-
MCCP	0.631	8.4	4.87	5.67	4.42	4.91	15.9	-

Maximum concentrations ($\mu\text{g/l}$) of chloroparaffins found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	0.326	1.84	1.29	1.22	4	1.14	4.3	4.75
MCCP	0.709	2.91	3.11	2.28	1.28	3.64	2.48	1.11

Maximum concentrations ($\mu\text{g/l}$) of chloroparaffins found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	0.014	10.4	2.47	2.04	0.71	0.97	10.9	1.11
MCCP	0.55	<0.60	<0.60	4.01	<0.60	4.11	21.0	3.28

Maximum concentrations (mg/kg d.w.) of chloroparaffins found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
SCCP	2.51	10.5	11.6	13.1	-	11.6	11.1	14.1
MCCP	37.6	1.23	2.35	<0.03	-	0.12	<0.03	2.27

Brominated flame retardants

Maximum concentrations (ng/l) of polybrominated diphenylethers found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	0.22	0.57	0.90	0.23	0.87	0.43	0.49	<0.5
octaBDE	<0.6	<0.15	1.5	0.08	0.17	<0.15	0.09	<0.1
decaBDE	23	2.3	2.0	1.1	8.8	5.0	3.0	<1

Maximum concentrations (ng/l) of polybrominated diphenylethers found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	0.89	0.39	<0.15	0.17	1.5	4.5	0.33	-
octaBDE	<0.57	<0.15	<0.15	<0.15	0.16	14	<0.03	-
decaBDE	1.8	2.8	0.26	0.83	1.9	10	1.2	-

Maximum concentrations (ng/l) of polybrominated diphenylethers found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	0.72	1.3	0.48	1.1	1.1	<0.15	0.65	0.31
octaBDE	1.1	<0.15	<0.15	0.21	<0.15	<0.15	0.32	<0.1
decaBDE	19	10	4.0	9.8	2.6	0.19	6.1	<1

Maximum concentrations (ng/l) of polybrominated diphenylethers found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	0.7	11	11	1.4	0.69	<0.15	32	0.88
octaBDE	<0.75	1.4	1.2	0.23	<0.15	<0.15	2.7	<0.1
decaBDE	41	3.7	5.4	1.8	1.4	<0.15	2.1	<1

Maximum concentrations ($\mu\text{g}/\text{kg}$) of polybrominated diphenylethers found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
pentaBDE	49	30	62	32	35	29	36	34
octaBDE	1.7	2.3	2.5	2.5	2.5	2.4	2.2	<0.1
decaBDE	590	1180	600	240	1080	240	450	9.9

Maximum concentrations (ng/l) of hexabromocyclododecanes found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -HBCD	-	1.3	0.95	8.3	2.3	0.8	0.62	-
β -HBCD	-	0.67	0.5	4.1	<0.10	0.37	0.4	-
γ -HBCD	-	2.5	2.5	56	18	2.2	3.1	-
SUM HBCD	1.0	3.5	3.5	68	20	3.4	4.1	7

Maximum concentrations (ng/l) of hexabromocyclododecanes found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -HBCD	-	0.71	0.42	0.92	0.43	32	0.43	-
β -HBCD	-	0.42	0.37	0.08	<0.10	7.3	0.29	-
γ -HBCD	-	1.6	3.1	2.9	0.46	73	1.2	-
SUM HBCD	<5	2.8	3.9	3.8	0.89	110	2.0	-

Maximum concentrations (ng/l) of hexabromocyclododecanes found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -HBCD	-	2.4	0.66	1.8	2.9	0.34	0.43	-
β -HBCD	-	0.95	<0.10	1.3	0.17	0.15	<0.01	-
γ -HBCD	-	3.9	0.39	68	3.1	0.46	1.1	-
SUM HBCD	<5.0	6.5	0.66	70	6.2	0.95	1.5	1.3

Maximum concentrations (ng/l) of hexabromocyclododecanes found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -HBCD	-	0.99	<0.10	<0.10	8.1	<0.10	<0.01	-
β -HBCD	-	0.08	<0.10	<0.10	55	<0.10	<0.01	-
γ -HBCD	-	<0.10	3.0	0.1	12	<0.10	3.3	-
SUM HBCD	<4.55	1.1	3.0	0.1	75	<0.10	3.3	<1

Maximum concentrations ($\mu\text{g}/\text{kg}$) of hexabromocyclododecanes found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
α -HBCD	-	97	9.8	24	72	89	9.6	-
β -HBCD	-	14	3.1	11	5	12	7.8	-
γ -HBCD	-	86	120	23	200	29	6.8	-
SUM HBCD	180	180	130	47	210	130	24	9.5

Perfluorinated compounds

Maximum concentrations (ng/l) of perfluoroalkyl acids found in municipal effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	25	1.1	640	5.2	1.7	1.3	15	26
PFOA	15	12	15	14	4.8	4.6	18	18
PFHxA	8.7	3.1	9.5	5.3	1.4	0.73	3.9	10
PFDA	5.7	4.2	1.4	1.4	1.1	1.3	1.2	2.1

Maximum concentrations (ng/l) of perfluoroalkyl acids found in industrial effluents.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	27	2	1300	0.31	1.3	2.2	1.5	-
PFOA	11	14	100	1.1	2.2	6.4	3	-
PFHxA	11	2	75	<0.5	1.1	4.1	1.1	-
PFDA	<5	<0.5	1.7	<0.5	1.2	<0.5	<0.5	-

Maximum concentrations (ng/l) of perfluoroalkyl acids found in storm water.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	420	1.5	9.9	3.7	0.69	3.9	0.6	6.9
PFOA	67	1.9	5.1	4.0	<0.5	2.9	1.1	48
PFHxA	180	0.79	17	1.8	<0.5	<0.5	<0.5	20
PFDA	5.4	<0.5	0.60	1.0	<0.5	0.90	<0.5	20

Maximum concentrations (ng/l) of perfluoroalkyl acids found in landfill leachate.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	<10	110	140	59	6.7	<0.5	370	1500
PFOA	130	590	270	220	26	1.4	710	2000
PFHxA	700	600	200	130	32	<0.5	820	2900
PFDA	<10	21	3.7	9.3	0.61	<0.5	200	5.6

Maximum concentrations ($\mu\text{g}/\text{kg}$) of perfluoroalkyl acids found in sludge.

	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
PFOS	45	3.0	110	14	1.8	3.5	23	28
PFOA	2.5	0.73	0.91	0.99	0.33	0.99	0.97	5.2
PFHxA	<2.4	0.16	0.72	0.22	0.22	<0.1	2.1	1.4
PFDA	16	2.7	0.97	4.7	1.6	2.2	3.2	5.3

Appendix B

Rationale of the whole effluent assessment (WEA)

Thousands of different chemicals are used in various sectors of the society (households, industry, hospitals etc.). The majority of these substances end up in sewage. Consequently, wastewater treatment plants are facing multiple challenges in their techniques and capacity. Unknown transformation products of chemical substances, either entering the treatment plant or produced there during the treatment process by microbial activity, may exhibit harmful properties. Some of the chemical compounds or their transformation products are persistent, bioaccumulating and potentially toxic. Waste water treatment techniques may not be optimized to tackle these compounds at low concentration but rather the main focus has been on the removal of nutrients.

Scientific knowledge about combined effects of chemicals is still rather limited. The majority of the knowledge about the toxic effects of chemicals is only valid for single compounds. From the environmental point of view exposure to a single compound is an artificial situation.

Hazardous substances are widely discharged to the Baltic Sea. The management of some common hazardous substances has been effective, but there are new threats caused by emerging, poorly known substances. Even though individual compounds were found at low concentrations, the mixture of many persistent compounds may lead to detrimental effects on the marine biota. The problem with policy instruments based on the single chemicals at a time is that they leave the discharges of many potentially hazardous substances uncontrolled and do not address the possibility of combined effects. Banning or restriction of the use of one hazardous substance does not always prevent from replacing it with another, equally hazardous substitute or with a substitute carrying unknown properties.

One goal of the COHIBA project was to track toxicity of effluents, leachates and storm waters entering into the Baltic Sea by using the whole effluent assessment (WEA). WEA means simply testing biological effects of the samples as such, preferable without any manipulations, or after as little manipulations as possible. In general, pH adjustment, aeration, or other minor pre-treatment may be unavoidable to assure the welfare of test organisms. WEA differs from the chemical compound-specific approach, because it is not limited to a pre-selected compound or group of compounds, but the test organisms are exposed to all the substances present in the sample.

The aim of the project was also to analyse 11 substances and substance groups assessed by HELCOM as priority substances. Traces of all the substances were detected. Although it is well known that these particular compounds are not acutely toxic to aquatic organisms at the observed concentrations, there were indications of toxic effects in the treated effluents.

By using WEA it is not possible to find out the causative chemical or mixtures of chemicals unless all the results were verified by similar tests using known chemicals and the same concentrations as had been in the original effluent samples. This would be an enormous task and practically impossible. However, further analyses using WEA in combination with chemical analyses and fractionation of the effluent sample it is possible to identify the hazardous substances (toxicity tracking), and hence, to plan necessary actions. This procedure should be an effective tool to increase the level of protection of the Baltic Sea and enhance its ecological status.

WEA and laboratory test comparison results were used to derive a proposal for toxicity-based discharge limits. The proposal is available at the HELCOM web pages. The principle of setting the toxicity limits was to ensure, that effluents discharged into the Baltic Sea should not cause acute toxicity and only moderate long-term toxicity by the recommended methods.

WP3 proposed limits for maximum allowable acute toxicity stated as follows: 30% inhibition of algae growth at 80% test concentration, 20 % immobility of *Daphnia magna* at 95% test concentrations (48 h exposure), and 30% inhibition of luminescent bacteria (30 min exposure) at 80 % test concentration. When toxicity test results are compared with these limit values, the COHIBA results indicated that the quality of municipal wastewater effluent varied greatly. The variation was apparent by both chemical and biological methods, since there were acute toxicity in some occasions. If the principle is accepted that acute toxicity should not be observable at all, the results should lead to further actions, and at least to confirm the results by repeated tests.

Because chemical and biological methods do not measure the same phenomenon, detailed cost comparisons are not well justified. However, based on the COHIBA WP3 work some comparison of the costs of toxicity tests and chemical analyses could be produced. Laboratory facilities for bio-testing are quite simple. Needed analytical equipments are relatively simple equipments, such as microplate readers, spectrophotometer, luminometer, and relevant facilities for culturing the test organisms. Pre-treatment of samples is simple; adjustment of pH, filtration or centrifugation and aeration. For chemical analyses sophisticated instruments e.g. GC and LC equipped with different identification and detection systems are needed. The pre-treatment of samples is intensive (analytical methods are described in the ring-test reports, www.cohiba-project.net). Instrument use and maintenance and laboratory chemicals should also be added to the costs of these analyses. Price of a test or analysis is comprised of personnel costs, investment on instruments and facilities, direct costs from reagents and consumables and the number of samples.

In the following tables comparison of toxicity tests and chemical analyses, performed by one partner in 2009 - 2011, is based on the laboratory working time. Only 5 groups of chemical analyses, not all of the 11 HELCOM priority substances, and only acute toxicity tests were taken into account.

Table shows the starting point of the comparison

Sample type	Number of samples	
	Toxicity tests	Chemical analyses
effluent	24	24
storm water	2	2
landfill leachate	2	2
sludge	0	6
total	28	34
	Tests	Analyses
	Luminescent bacteria D. magna immobilization Algae growth	Cd PBDE HBCD PFOS, PFOA Phenolic substances

Toxicity tests	Chemical analyses
28 samples	34 samples
36 days	156 days
3 tests	5 substance groups
time/sample 1,3 d	time/sample 4,6 d

Chemical and biological methods do not answer the same question, and both methods have their advantages and limitations. Therefore, it is not well justified to compare the costs of the methods. The protection of the Baltic Sea is not a question of the type of the effluent monitoring methods, only the quality of the effluent matters. So, prevention of further pollution of the Baltic Sea, new and precautionary approaches in the chemicals control are necessary. By only measuring chemical concentrations of a rather limited number of substances released to the environment, the Baltic Sea undisturbed by hazardous substances (in BSAP) will hardly ever be achieved.

COHIBA (Control of Hazardous Substances in the Baltic Sea) is a joint project of Baltic Sea countries in 2009 - 2012. The eleven substances or substance groups identified in the HELCOM Baltic Sea Action Plan (BSAP) as being of special concern to the Baltic Sea are the focus of this project.

This is the summary report of COHIBA WP3 work. The principal goals of WP3 were to identify sources of hazardous substances discharged into the Baltic Sea, and to assess the effluent quality by biological methods (Whole Effluent Assessment, WEA).



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