Final Report

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STUDY ON ENDOCRINE DISRUPTERS IN DRINKING WATER

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Schmallenberg and Wiesbaden, February 26, 2003

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SUMMARY

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption requires in Article 7 from Member States that they "shall take all measures necessary to ensure that regular monitoring of the quality of water intended for human consumption is carried out, in order to check that the water available to consumers meets the requirements of this Directive and in particular the parametric values set in accordance with Article 5." The chemical parametric values are specified in Annex I, part B. The list - among others – comprises 26 parameters. These, however, do not include parameters for endocrine disrupting properties. Recital 15 clearly states that "there is at present insufficient evidence on which to base parametric values for endocrine disrupting chemicals at Community level".

Besides the main exposure route – the uptake via food - humans can in theory be exposed by the consumption of drinking water. However, currently the exact concentrations of endocrine disrupting compounds in drinking water and thus the consumed quantities are currently uncertain for all the European countries.

The aim of this study was to provide information on the exposure to endocrine disrupters through water intended for human consumption, using existing data from the various Member States and literature studies, and to design and execute a case study to produce a more complete picture of the actual occurrence of endocrine disrupters and the potential exposure of humans to endocrine disrupters through drinking water.

The following EDCs were selected as relevant for drinking water from an assessment of the published literature and a survey of waterworks of several European countries:

Synthetic and natural estrogens, alkylphenols, alkylphenol ethoxylates, alkylphenoxy acetic acids, Bisphenol A and organotin compounds.

These compounds were analysed in raw and drinking water sampled at 4 European waterworks. Pesticides were not analysed in the case study. For the evaluation, pesticide data reported in the questionnaires and in literature were used.

Based on the case study, the literature survey and the evaluation of questionnaires filled in by the waterworks, the results can be summarised as follows.

Pesticides

Pesticides which have the potential for endocrine disrupting activity can be present in groundwater and raw water used for drinking water production at varying frequencies and concentrations. Based on the available data, drinking water samples with concentrations exceeding 0.1 µg/L were reported in a few cases. Comparable to surface water and groundwater, most information for drinking water were available for the pesticides Atrazine, Simazine, Diuron, Isoproturon and Lindane.

Since the limit values of the drinking water directive for pesticides are lower than potential guidance values based on human health, direct health hazards via the reported concentrations in drinking water are not expected. However, these guidance values are not available for all pesticides.

Biocides: Organotin compounds

Of all the organotin compounds TBT is of most concern. TBT was detected in surface waters up to 0.05 µg/L. No data were available regarding organotin compounds and especially TBT in groundwater and drinking water. In the case study, trace amounts (ng/L) of MBT, DBT and DMT were found in raw water. These compounds are typically used as stabilisers in plastics. TBT was detected neitehr in final drinking water nor in tap water investigated in the case study. However, this should be verified on an European level.
Due to its physico-chemical properties TBT sorbs onto particles and should generally be removed by passage through soil or sediment to groundwater and by the respective drinking water treatment processes. Nevertheless, leaching from plastic products such as pipes can lead to TBT concentrations in the treated water.

Based on a worst case scenario for human exposure towards TBT it seems to be unlikely, that drinking water can significantly contribute to an organotin exposure. Therefore, a direct health hazard via drinking water is not expected.

Environmental chemicals

Bisphenol A

Bisphenol A can be present in surface waters in the upper ng/L range. Groundwater data are lacking so far. Bisphenol A was reported to be present in raw water and drinking water in a few cases in the lower ng/L range. In the tap water samples of the case study Bisphenol A could not be detected. Some reported controversial results from the literature needs further verification.

It can be concluded from the existing data, that drinking water contributes to a minor part to human exposure to Bisphenol A. Based on the current assessment on human health effects, the Bisphenol A quantity consumed via drinking water seems to be unlikely to pose a risk for humans.
Due to the limited information on Bisphenol A concentrations in raw water and drinking water, the database should be enlarged to enable a European wide picture.

Alkylphenols

Alkylphenols were frequently detected in surface waters. Nonylphenol occurs in the µg/L-range, Octylphenol was detected at lower concentrations in the ng/L range. The presented data

imply a great variation of Nonylphenol concentrations in European surface waters due to varying efficiencies of the purification processes in the sewage treatment plants.
For groundwater and drinking water not much information is available. The reported concentrations for Nonylphenol and Octylphenol are in the ng/L range; only in one case 2 µg/L were reported. Tap water samples analysed in the case study contained neither Nonylphenol nor Octylphenol above the limit of quantification.
It can be concluded from the existing data, that drinking water contributes to the exposure of humans to alkylphenols only to a minor part.

Based on the current assessment concerning effects on humans, the Nonylphenol quantity consumed via drinking water seems to be unlikely to pose adverse health effects.
However, in order to determine drinking water standards and to evaluate the relevance of the presented Nonylphenol concentrations for humans, more toxicological data as well as more reliable drinking water concentrations of Nonylphenol are essential.

Synthetic and natural estrogens

Synthetic estrogen 17α-Ethinylestradiol
17α-Ethinylestradiol can occur in surface waters at concentrations of a few ng/L. There are limited data regarding the occurrence in groundwater. In drinking water 17α-Ethinylestradiol was detected neither at waterworks nor in the samples of the case study. Low concentrations around 1.4 ng/L reported in the literature seems to be outliers, thus further confirmation is needed.

The contribution of drinking water to the exposure of humans towards 17α-Ethinylestradiol seems to be very low. However, since 17α-Ethinylestradiol is one of the most potent synthetic estrogenic compounds unintentional exposure of the population, especially developing embryos or prepubertal boys and girls, should strictly be avoided.
For an assessment of the exposure of humans via drinking water in Europe, more reliable data on the concentrations in raw water and drinking water are required.

Natural estrogens: 17β-Estradiol and Estrone
17β-Estradiol can be present in surface waters in the lower ng/L range and in groundwater below 1 ng/L.
In the case study generally no 17β-Estradiol and Estrone were detected. In literature and questionnaires from waterworks Estrone concentrations in drinking water up to 1 ng/L were reported. Due to the treatment train of the waterworks investigated, this high concentration is not plausible and needs confirmation.

Compared to an average dietary intake of natural estrogens via food, the roughly estimated ingestion of estrogens with drinking water using a worst case scenario seems to contribute with a maximum of around 4 % to the total exposure of an adult via mixed diet.
Based on this knowledge, effects on humans due to the intake of steroid hormones in drinking water are not expected. However, for an evaluation of an European wide situation more reliable data are required.

Biological analyses using the Yeast Estrogen Screen (YES assay)
The findings of the bioassay qualitatively confirmed the results of the chemical analyses. Hence, the bioassay was very sensitive and underlined the positive results of the chemical analysis, where natural estrogens were found in one raw water sample. All other samples, including drinking water and tap water, were negative in the bioassay indicating that estrogenic activity was not present above the limit of quantification of 0.01 and 0.05 ng/L 17α-Ethinylestradiol equivalents.

The following résumé can be drawn from the results of the project:

- The raw water of waterworks can be contaminated by EDCs. This is especially the case if surface water is used as water resource.
- Depending on the water treatment drinking water might contain EDCs at very low concentrations. However, the few positive results available from literature are not finally confirmed due to the lack of standardised analytical methods.
- Therefore, prior to a European wide monitoring of drinking water which seems to be essential for a final conclusion, the analytical methods for the analysis of EDCs in drinking water should be standardised.
- Efficient drinking water treatment trains are appropriate to avoid a drinking water contamination by EDCs (e.g. using ozonation, activated carbon, bank filtration).
- Even if the raw water of waterworks is free of EDCs, drinking water/tap water might be contaminated by individual EDCs due to leaching from materials used in waterworks and water pipes.
- Even if the highest concentration of an individual EDC reported for drinking water is considered for the assessment of effects on humans, based on the current knowledge, endocrine effects via the consumption of drinking water are very unlikely.

CONCLUSION

On the basis of a literature survey, a European wide questioning of waterworks and a case study on the occurrence of EDCs in four European waterworks, the following conclusions can be drawn concerning

- **Occurrence of EDCs in raw water and drinking water**:

  ⇒ *In the literature and in the questionnaires sent to waterworks and authorities* the presence of EDCs in drinking water were reported. Some of the listed concentrations were relatively high. Although detailed analytical protocols were not available, it can be assumed that these are outliers. Therefore, further measurements with standardised analytical methods are crucial for confirmation.

  ⇒ *In the case study* where drinking water was analysed at 4 European waterworks for synthetic and natural estrogens, alkylphenols, bisphenol A and organotin compounds, concentrations were below the limit of quantification. Only in a few cases very low EDC levels close to the quantification limit could be detected.

  ⇒ Most information about the presence of EDC concentrations in raw water and drinking water were available *from a limited number of European countries*. These results cannot be transferred to all Member States of the European Union. To provide a union wide picture of the exposure additional data from other European countries are required.

  ⇒ For pesticides most frequently the occurrence of Atrazine, Simazine, Diuron, Isoproturon, Linuron, Endosulfan and Lindane was reported in raw water and drinking water. In a few cases even the limit value of the Drinking Water Directive for individual pesticides was exceeded.
  For the other pesticides selected for this study no information on drinking water concentrations was available.

  ⇒ The raw water of waterworks, especially surface waters, frequently contains EDCs. However, common drinking water treatment technology (e.g. bank filtration, coagulation, ozonation, GAC) should be very effective in removing EDCs. This is underlined by the results of the case study, the literature and by novel results from the EU research project POSEIDON (EVK1-CT-2000-00047).

Following conclusions can be drawn with respect to

- **Human exposure via drinking water**

  ⇒ The evaluation of the human exposure routes for EDCs investigated in the case study revealed that the contribution of drinking water to the total exposure is of minor importance for these substances.
  The main exposure route for these substances and for the pesticides is food consumption. Exceptions might occur for synthetic hormones used as pharmaceuticals. However, synthetic hormones such as Ethinylestradiol were detected neither in raw waters of waterworks nor in drinking waters.

  ⇒ The reported drinking water concentrations are clearly lower than drinking water guideline values (TBT) or "provisional drinking water guidance values" derived from TDI values (Bisphenol A) and animal studies (Nonylphenol).

  ⇒ Even if worst case contaminations of EDCs reported in the literature or in the questionnaires were considered for an assessment, a direct health hazard due to the consumption of drinking water contaminated with the individual EDC is not expected using the currently available toxicological data and existing TDI values.

  ⇒ According to current knowledge, for the selected pesticides, endocrine effects on humans due to the consumption of drinking water containing the maximum concenration level of 0.1 µg/L for individual pesticides are very unlikely.

IDENTIFICATION OF GAPS OF KNOWLEDGE

- The preceding conclusions were based on data from a limited number of European Countries. Therefore, for final conclusions regarding the European wide exposure of consumers to endocrine disrupters through drinking water, the database is too small. More data from a greater number of waterworks in Europe are required.

- In the case study only a limited set of highly relevant substances could be analysed. Information on other drinking water contaminants with potential endocrine activity is limited. Therefore, additional studies on the occurrence of further endocrine disrupting compounds should be performed.
  It should also be examined, whether pesticides other than the most frequently measured ones exceed the limit value of the Drinking Water Directive.

- Due to the limited set of analytical data for EDCs in drinking water available from the literature and the returned questionnaires, it cannot be excluded that sources with higher levels of exposure than determined in this study exist. Therefore, further analysis and research is needed to elucidate contradictory results comparing analytical data reported in the literature, data from the questionnaires and currently available data on the effectiveness of water treatment.

- The removal efficiency of the water treatment processes should be carefully checked to minimize a potential drinking water contamination by EDCs, and to enable the estimation of the occurrence of EDCs. There is a need for research on the removal efficiency of different water treatment processes in order to better understand the removal of potential EDCs.

- The approval process for materials used in waterworks and water pipelines should take into account a potential release of EDCs from additives used in these materials.

- Analytical methods for EDCs in drinking water should be standardised before starting further measuring programmes.

1 INTRODUCTION

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption states in recital (15) that "there is at present insufficient evidence on which to base parametric values for endocrine disrupting chemicals at Community level, yet there is increasing concern regarding the potential impact on humans and wildlife of the effects of substances harmful to health".

However, a consideration of suspected endocrine disrupters is needed following precautionary principles though "a causative role of these chemicals in diseases and abnormalities (possibly related to an endocrine disturbance) remains uncertain. Assessed from relative potencies based on oestrogen receptor interactions, steady-state concentrations of endocrine disrupting chemicals in normal human serum concentrations in drinking water, even in a worst case, are several orders of magnitude lower than those of endogenous hormone levels. However, a number of the compounds implicated do not exert their effects via the oestrogen receptor" (CSTEE 1999).

Besides the main exposure route - that is the uptake via food - humans could potentially be exposed through the consumption of drinking water. However, currently the concentrations of significant EDCs in drinking water are uncertain and the exposure from this source is not known for the countries of the European Union.

In response to the above the Commission undertook to fund a study on potential and known EDCs in drinking water. The study aims at the assessment of the exposure of consumers to potential endocrine disrupters through drinking water in order to determine the extent of exposure and to provide information for risk assessment.

2 SUBJECT AND SCOPE

Within EU Member States some data and further information of varying status, quality and density exist on the sources, occurrence and concentrations of industrial chemicals, pharmaceuticals, plant protection products, and unintentionally formed substances with (suspected) endocrine disrupting properties in drinking water and raw water used for the production of drinking water. The study was intended to provide a better basis for interpreting existing information. In order to select the key potential EDCs for the study, a clear definition of „endocrine disrupter“ was needed. For this purpose the following definition given in the call for tenders was used:

„A potential endocrine disrupter is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations.

An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)population“.

(Communication from the Commission to the Council and the European Parliament – Community Strategy for Endocrine Disrupters COM(99)706).

The subject and scope of the study as given in the technical annex of the call for tender were as follows:

1. to provide a union wide picture of the exposure to endocrine disrupters through water intended for human consumption, using existing data from the various Member States;

2. to identify gaps in knowledge on the presence, concentrations and persistence of endocrine disrupters in drinking water and, also, raw water used for the production of drinking water;

3. to design and execute a pilot study in selected areas of the EU to produce a more complete picture of the actual occurrence of endocrine disrupters and the exposure of human beings to endocrine disrupters through drinking water;

4. to report on the known and potential effects on human health attributable to the presence of such endocrine disrupters;

5. to identify those individual endocrine disrupters or groups of endocrine disrupters that might be appropriate for future regulation in drinking water.

Furthermore it was a central scope of the study to

6. establish an EU-wide information network on endocrine disrupting chemicals in drinking water and raw water; participants were representatives from water suppliers, EUREAU, scientists dealing with endocrine effects, and representatives from Commission services concerned. These discussions would support efficient work and optimised the presentation of the elaborated information with regard to the final customers of the study and assured the required degree of transparency.

Thus, the final subject of the work was in fact twofold:

- assessment of the union wide exposure of consumers to selected endocrine disrupters through drinking water intended for human consumption,
- provide data for a risk assessment of endocrine disrupters for drinking water consumers.

The study, which aimed at the identification and investigation of known and potential endocrine disrupters in water intended for human use, was divided into two phases. Phase 1 was a desk study to develop an inventory of existing data in the EU-Member States. Phase 2 comprised the design and execution of the case study based on the results from Phase 1.

Phase I: Desk study, inventory of existing data

- Selection of EDCs relevant for drinking water
- Questionnaires for waterworks and authorities for the selection of existing monitoring data
- Organisation of an information network
- First Evaluation

Phase II: Design and execution of the case study

- Selection of EDCs for the chemical analyses
- Design of the sampling programme and selection of the waterworks
- Executions of the field study (chemical and biological analyses)
- Evaluation
- Identification of gaps of knowledge

3 PERFORMANCE OF THE PROJECT

3.1 Inventarisation of existing data: Phase I

3.1.1 Literature survey

For the literature search following databases were used:

Medline/Toxline	Dimdi	http://www.dimdi.de/de/db/recherche.htm
PUBMED	NCI	http://www.ncbi.nlm.nih.gov/Entrez/index.html
Science Direct	Elsevier Science	http://www.sciencedirect.com
Current Contents	Ovid	CD, owned by the Fh-IME.
Toxline	National Library of Medicine	http://toxnet.nlm.nih.gov/

The search focused on recent articles published later than 1995. Furthermore, common search engines (e.g. google) and the homepages of several member states of the EU and of the European Union, WHO and JRC were used.

3.1.2 Selection of endocrine disrupting substances relevant for drinking water

So far, more than 600 chemicals have been discussed as potential endocrine disrupters. It is very well established that there is no direct link between *in vitro* information and *in vivo* effects. For the purpose of the study the definition of the Communication from the Commission to the Council and the European Parliament – Community Strategy for Endocrine Disrupters COM(99)706 – was followed (see page 2).

The criteria for substance selection were:

- (potential) endocrine disrupting activity based on *in vitro* and *in vivo* effects reported in literature
- occurrence in surface water, which is frequently used as a source of raw water for drinking water production
- occurrence in groundwater or potential for reaching groundwater which is frequently used as raw water for drinking water production, with minimal treatment.

For the compilations of selected substances international organisations and authorities were considered where possible (OSPAR 1998, Commission of the European Communities (COM 1999), UK Environmental Agenda List, National Toxicology Program NTP 2000). The selected substances with relevance for drinking water and their presumptive endocrine disrupting mechanisms are listed in Table 1.

Biocides

Tributyltin oxide or the tributyltin cation, respectively, has been used mainly as an antifouling paint on vessels but also in small amounts for the protection of materials, e.g. gaskets. Since 1990, the use of TBT as antifouling paint for vessels is limited to ships with hulls greater than 25 m (Directive 89/677/EEC). A restriction for the use of TBT in antifouling paints for all boats on inland waters became effective in 2000. Because the PVC stabiliser DBT contains TBT as technical contaminant at concentrations up to 1 % (based on Sn) (German UBA 2000), TBT can enter the aquatic environment e.g. via leaching from newly installed plastic pipes. From the group of organotin compounds TBT is of most concern because it is a strong aromatase inhibitor, responsible for endocrine disrupting activity in molluscs by prolonging the half-life of testosterone in the organism, and it is of higher immunotoxicity than the other organotin compounds. o-Phenylphenol is used as preservative (post harvest mold and rot control e.g. for the treatment fruits). Due to its phenol structure the substance is able to activate the estrogen receptor. It can enter the aquatic environment via domestic waste water and leachates from deposits for domestic waste (Ternes et al., 1998).

Pesticides

Out of the 22 pesticides 18 substances were classified as substances which have a potential for endocrine activity from the Commission of the European Union (COM 1999) and OSPAR (1999). These substances are marked with the reference number 10 and 3, respectively, in Table 1. Methoxychlor and synthetic pyrethroids are discussed by OSPAR as suspected endocrine disrupters. Regarding Methoxychlor, classic estrogenic activity is reported to occur in F1 rats following in utero and perinatal exposure to 5 mg/kg/day or higher (NTP 2000). Isoproturon with suspected anti-androgen activity was included in the questionnaire due to safety reasons. Though the endocrine activity is questionable or there are insufficient data, respectively, Heptachlor and Trifluralin were selected for the questionnaire because they had been detected in surface waters (COMMPS 1999).

Environmental chemicals

Out of the environmental chemicals selected for this study 16 were also classified by the Commission of the European Union (COM 1999, ref 10 in Table 1) and/or OSPAR (1999, ref 3 in Table 1) as substance with the potential for endocrine activity. Benzophenone, used as UV-stabiliser and fragrance enhancer in cosmetics, was included due to its estrogenic activity (Miller et al., 2001). Environmental chemicals, often also called environmental estrogens or xenoestrogens, are rather weak hormone mimics in comparison with endogenous steroid estrogens and potent estrogenic drugs (Degen et al. 2002, Mäkelä et al. 1999).

Hormones, drugs and phytoestrogens

Tamoxiphen and 17α-Ethinylestradiol are synthetic steroids with anti-estrogenic and estrogenic activity, respectively. Tamoxiphen is used in the treatment of breast cancer and 17α-Ethinylestradiol is the most common used contraceptive.
17β-Estradiol is the main natural estrogen synthesised by all vertebrates. It is partly metabolised in the body to Estrone and Estriol. Testosterone is the natural androgen synthesised by all vertebrates.
The natural estrogens are also used as pharmaceutical for climacteric disorders. The pharmaceuticals and natural estrogens are released into the aquatic environment mainly via the sewer systems. But the natural estrogens and androgens can also reach surface waters via run-off from grass land with cattle breeding or contamination with liquid manure.

Phytoestrogens

Phytoestrogens are plant derived compounds with estrogenic activity which include the chemical classes of isoflavones, lignans, coumestanes and indole-3-carbinole. Important phytoestrogens for humans are the isoflavones Daidzein and Genistein, with the highest quantities found in soybeans, soy milk and other soy protein products (Degen et al. 2002). Daidzein and Genistein are less potent estrogens in comparison with endogenous steroid estrogens but more potent than the environmental chemicals. Equol is a metabolite of Daidzein and used as a drug against climacteric disorders. Since the isoflavones are food components or ingested as pharmaceutical they can reach the aquatic environment via sewer systems. β-Sitosterol is a steroidal plant compound of many plants and is used in minor quantities as lipid regulator in medicine. Environmental entry occurs via sewage systems and plant residues. β-Sitosterol and Genistein (Kiparissis et al., 2001) are also present at high concentrations in paper mill effluents. β-Sitosterol has estrogenic activity and reduces reproductive fitness in fish and sperm counts in rats. The phytosterol Stigmasterol had also been detected in surface waters (Ternes et al. 2001) and was included into the questionnaire due to structural analogy to β-Sitosterol.

3.1.3 Questionnaires

3.1.3.1 National authorities

Questionnaires were sent to national authorities of the Member States of the EU (questionnaire see Appendix A 1.1, page 89). The addresses were taken from the homepage of the European Environment Information and Observation Network (EIONET, see references), a collaborative network of the European Environment Agency in Copenhagen (EEA) and its member countries. The questionnaires were sent to the focal points of 15 European countries.

3.1.3.2 Waterworks

The questionnaires for waterworks were available in 4 languages (English, Dutch, French, German, see A 1.2, page 93) and could be downloaded from the homepage of the European project Poseidon (EU-Poseidon.com), the homepages of the ESWE-institute (www.uni-mainz.de/~) and of the Fraunhofer Institute (www.ime.fraunhofer.de/download/SET). Furthermore, the questionnaires were announced and distributed on the EUREAU meeting of January 2001 in Geneva to national representatives of most of the European countries.

Table 1: Selected substances with relevance for drinking water and their presumptive endocrine disrupting mechanisms

Substance	CAS Number	Propable Mechanism in vitro or in vivo, for details see references
Biocides		
TBT	-	androgenic (Aromatase Inhibition) [3, 10]
TBTO	56-35-9	androgenic (Aromatase Inhibition) [10]
o-Phenylphenol	90-43-7	estrogenic [1, 10]
Pesticides		
Acetochlor	34256-82-1	estrogenic [2, 10]
Alachlor	15972-60-8	estrogenic, weak, TSH serum level increase [10]
Amitrol	61-82-5	(potential) endocrine [10]
Atrazine	1912-24-9	estrogenic [3, 10]
Chlorfenvinphos	470-90-6	estrogenic, but questionable [4], insufficient data [10]
Dicofol (Kelthane)	115-32-2	endocrine in-vitro [3, 10]
Diuron	330-54-1	no effect [3], but metabolite 3,4-DCA anti-androgenic [10]
Endosulfan	115-29-7	estrogenic [3, 10]
Heptachlor	76-44-8	estrogenic, but questionable [4], POP see below
Isoproturon	31412-59-6	suspected anti-androgenic [5] (conclusion by analogy to phenyl-urea), at high doses impairs androgen biosynthetic processes [6]
Lindane (gamma HCH)	58-89-9	endocrine [3, 10]
Linuron	330-55-2	androgenic, weak in-vitro, highly effective in-vivo [7, 8, 10]
Maneb	12427-38-2	(potential) endocrine [10]
Metam Sodium	137-42-8	(potential) endocrine [10]
Methoxychlor (active metabolite)	72-43-5	endocrine in-vitro, [3,] estrogenic in vivo [8]
Nitrofen	1836-75-5	(potential) endocrine [10]
Pyrethroids, synthetic; miscellaneous	-	endocrine in-vitro [3]
Simazine	122-34-9	estrogenic, structure analogy with Atrazine: endocrine in-vitro [3, 10]
Thiram	137-26-8	(potential) endocrine [10]
Trifluralin	1582-09-8	insufficient data [10]
Vinclozolin	50471-44-8	antiandrogen [8], endocrine in-vivo [3, 8, 10]
Zineb	12122-67-7	(potential) endocrine [10]
Environmental chemicals		
Benzo(a)pyrene (PAH)	50-32-8	endocrine in-vitro [3]
Benzophenone	119-61-9	estrogenic [9]
Bisphenol A	80-05-7	estrogenic, endocrine in-vivo [3, 10]
Chlordecone (Kepone)	143-50-0	endocrine in-vivo [3, 10], (obsolete, suspended world-wide)
3,4-Dichloroaniline	95-76-1	endocrine in-vivo [3, 10]
HCH beta	319-85-7	endocrine in-vivo [3]
4-Nitrotoluene	99-99-0	(potential) endocrine [10]
4-Nonylphenol (branched)	84851-15-3	estrogenic [3, 10]
4-p-Nonylphenol	104-40-5	estrogenic
Nonylphenol (techn. mixture)	25154-52-3	estrogenic [3, 10]

continued

Table 1: continued: Selected substances with relevance for drinking water and their presumptive endocrine disrupting mechanisms (continued)

Substance		**CAS Number**	**Mechanism (potential) in vitro or in vivo, for details see references**
Nonylphenol ethoxylate	NP1EO NP2EO	27986-36-3 9016-45-9	estrogenic [3, 10]
4-tert-Octylphenol		140-66-9	estrogenic [3, 10]
Phenolic compounds, e.g.			
4-tert-Butylphenol		98-54-4	estrogenic [3, 9, 10]
Resorcinol		108-46-3	(potential) endocrine [10]
Styrene		100-42-5	(potential) endocrine [10]
Hormones/ Drugs/ Phytoestrogens			
Tamoxiphen		10540-29-1	anti-estrogenic
17α-Ethinylestradiol		57-63 -6	estrogenic [3]
17β-Estradiol		50-28-2	estrogenic [3]
Estrone		53-16-7	estrogenic [3]
Estriol		50-27-1	estrogenic
Testosterone		8-22-0	androgenic
β-Sitosterol		83-46-5	weak estrogenic[13], decreasing testosterone levels in fish[14], reduction of sperm count in rates [15]
Daidzein		486-66-8	estrogenic
Equol		-	estrogenic
Genistein		446-72-0	estrogenic
Stigmasterol		83-48-7	suspected, analogy to β-Sitosterol (phytosterol)

**** POPs** (Persistent Organic Pollutants):

Aldrin	-
Chlordan,	endocrine in-vitro [3, 10] (obsolete in EU)
Dieldrin	endocrine in-vitro [3]
Dioxine,	endocrine in-vivo [3, 10]
DDT	endocrine in-vivo [3]
Endrin,	
Furane (PCDFs),	endocrine in-vivo [3, 10]
Heptachlor,	ER agonist, but questionable,
Hexachlorobenzene	endocrine in-vitro [3, 10] (obsolete in EU)
PCBs	endocrine in-vivo [3] and hydroxymetabolites, banned
Toxaphene	endocrine in-vitro [3, 10] (obsolete, suspended worldwide)

[1] Sonnenschein, C., Soto, A. M (1998)
[2] Zeljenkova D, Vargova M (1996)
[3] OSPAR 1998, List of Candidate Substances (www.ospar.org)
[4] Vinggaard, A. and Breinholt et al. (1999)
[5] Stahlschmidt-Allner et al. (1997)
[6] Sarkar, S.N. et al. (1997)
[7] UK Environmental Agenda List: Endocrine-disrupting substances in the environment: The Environment‘ Agency’s strategy
[8] National Toxicology Program’s Report of the Endocrine Disruptors Low Dose Peer Review. October 10 - 12, 2000
[9] Miller, D., B.B. Wheals, N. Beresford, J. Sumpter (2001)
[10] Commission of the European Communities (COM 2001)
[11] More et al. (2001)
[12] Gray, L.E., Jr. (1998)
[13] Mellanen et al. 1996)
[14] MacLatchy and Van der Kraak (1994)
[15] Moghadasian (2000)

3.1.4 Organisation of an information network

As a means of building confidence, for exchange of information and discussion of results, a project-accompanying EU-wide information network was established. The participants were representatives from water suppliers, EUREAU, industry, national authorities and Commission services. The discussions were of considerable value for the progress of the project, especially with respect to the selection of the waterworks for the case study, and contributed to optimising the presentation of the information collected for the final customers of the study. Moreover, the required degree of transparency was assured. Two network meetings were held at April 16, 2002, and October 16, 2002, in Brussels. All participants of the network meeting had a chance to comment the draft of the final report. Many recommendations were then considered in the final version. The participants of the network are listed in Appendix 3.

3.2 Execution of the case study: Phase II

3.2.1 Selection of the substances for the case study

Based on the literature evaluation of the occurrence of EDCs, the following EDCs were selected due to their frequency of occurrence and their endocrine activity: *natural estrogens, contraceptives, phytosteroids, Bisphenol A, organotin compounds, alkylphenol ethoxylates and their breakdown products, alkylphenols and alkylphenoxy acetic acids*.
Though there was a variety of other compounds of concern, the number of substances for the case study had to be limited due to the temporal and financial limitations of the project.
It was agreed to exclude pesticides from the case study, because they are regularly monitored by waterworks and the information should be available for the respective authorities. Additionally, pesticides are regulated in the Drinking Water Directive. It was decided, to ask the waterworks monitored in the case study for providing their respective pesticide data.
Phthalates were excluded from the case study, since the special conditions for a reliable analyses (preparation of equipment, plastic free laboratory) would have enhanced the project efforts substantially.

In the following, the substances selected for the case study are shortly described:

Though the natural and synthetic estrogens occur only at low concentrations in surface waters, they are important contributors to the total estrogenic activity of water samples due to their extreme high potency.
Out of the phytoestrogens selected for the questionnaire, the phytosteroids β-Sitosterol and Stigmasterol were chosen, because they occur at higher concentrations in surface water than the flavonoids Daidzein and Genistein. Since Daidzein and Genistein are components of soy food they are released by sewer systems in low concentrations (Ternes et al., 2002).
Cholesterol, Coprostan-3-ol were included in the measuring programme as faecal indicator for sewage.

The organotin compounds were chosen since they were frequently detected in surface waters and TBT is of most concern due to its endocrine disrupting activity in molluscs by the inhibition of aromatase. Besides its use as biocide, TBT can be released from PVC pipes because DBT, used as PVC stabiliser, is known to be contaminated with TBT (German UBA 2000).
The other organotin compounds were included in the measuring programme as marker for organotin contamination.

Bisphenol A is mainly used for the production of epoxy resins and polycarbonates and is a weak estrogen.

Nonylphenol and Octylphenol are frequently detected in the environment at varying concentrations. The alkylphenols are weak estrogenic. For the other breakdown products of the alkylphenolethoxylates lower estrogenicity than for alkylphenols was reported.

Additionally, iodinated X-ray contrast media, which have no endocrine disrupting properties, were monitored to confirm the presence of treated municipal wastewater in the sampled waters.

Table 2: Substances selected for the case study

Substance		Occurrence
Natural estrogens and contraceptive		
Estrone		Sexual hormone, natural estrogen, metabolite of 17β-Estradiol
17β-Estradiol		Sexual hormone, natural estrogen
Mestranol		Pharmaceutical, contraceptive, synthetic estrogen
17α-Ethinylestradiol		Pharmaceutical, contraceptive, synthetic estrogen
17β-Estradiol-17-valerate		Pharmaceutical, Synthetic estrogen, climacteric disorders
Phytosteroids		
Cholesterol		Ingredient of cell membranes, red sea algae, zooplankton, fecal steroid, indicator for sewage
Cholestan		Basis for all steroids
Coprostan-3-ol		Microbial metabolite of cholesterol, fecal steroid, indicator for sewage
Campestrol		Ingredient of plant cell membranes
Lanosterol		Ingredient of non-photosynthesised plants, fungies, yeasts, Intermediates of squales, biosynthesis
17β-Sitosterol		Ingredient of plants (e. g. wheat sprouts)
Stigmasterol		Ingredient of plants (e. g. soya beans)
Organotin compounds		
Monomethyltin	MMT	Stabiliser in plastics materials
Dimethyltin	DMT	Stabiliser in plastics materials
Trimethyltin	TMT	Stabiliser in plastics materials
Monobutyltin	MBT	Stabiliser in plastics materials, surface coatings, catalysts
Dibutyltin	DBT	Stabiliser in plastics materials, surface coatings, catalysts
Tributyltin	TBT	Biocide
Tetrabutyltin	TTBT	Chemical industry
Monooctyltin	MOT	Stabiliser in plastics materials
Dioctyltin	DOT	Stabiliser in plastics materials
Bisphenol A		Plastics industry (production of polycarbonate and epoxy resins)
Alkylphenols and alkylphenol ethoxylates *		
4-*tert*.-Octylphenol	4tOP	Decomposition product of 4-*tert*.-Octylphenol-(poly)-ethoxylates
4-*tert*.-Octylphenol monoethoxylate	4tOP1EO	Decomposition product of 4-*tert*.-Octylphenol (poly)-ethoxylates
4-*tert*.-Octylphenol diethoxylate	4tOP2EO	Decomposition product of 4-*tert*.-Octylphenol (poly)-ethoxylates
4-Nonylphenol (technical isomer mixture)	4NP	Decomposition products of 4-Nonyl phenol(poly)-ethoxylates
4-Nonylphenolmonoethoxylat (technical isomer mixture)	4NP1EO	Decomposition products of 4-Nonyl phenol(poly)-ethoxylates
4-Nonylphenol diethoxylate (technical isomer mixture)	4NP2EO	Decomposition products of 4-Nonyl-phenol(poly)-ethoxylates
4-*tert.*-Octylphenoxy acetic acid	4tOP1EC	Transformation product of 4-*tert.*-Octylphenol polyethoxylates
4-Nonylphenoxy acetic acid	4NP1EC	Transformation product of 4-Nonylphenol polyethoxylates

*) 4-Nonyl-phenol (poly)-ethoxylates and 4-*tert*.-Octylphenol (poly)-ethoxylates are used among other things in the production of tensides, emulsifying agents, synthetic resins or flexibilisers.

3.2.2 Selection of waterworks

From 4 European countries 4 waterworks were selected following consideration of two aspects: a) the influence of different raw water sources and b) a wide variety of different treatment processes. Furthermore, the waterworks should be reached within 1 day. Due to temporal and financial limitations only 4 waterworks could be included in this pilot study.

Waterworks I used river water known to have a significant percentage of treated municipal sewage.

Waterworks II was fed by seapagewater from a rural area with a high density of livestock.

Waterworks III used canal water which was mainly influenced by municipal STW discharges.

Waterworks IV used deep groundwater pumped out of the second vertical aquifer layer of an area where treated wastewater is used to recharge the aquifer through irrigation of fields used for agriculture. The treatment processes used in the waterworks were: slow and fast sand filtration, flocculation/coagulation, granular activated carbon (GAC) filtration, ozonation and chlorination. The individual treatment trains are listed below:

- Waterworks I: intake of raw water from a large river into a storage reservoir, slow sand filtration, ozonation, flocculation, GAC, chlorination;
- Waterworks II: seapagewater from a polder, coagulation, residence time in a lake (3 month), rapid sand filtration, ozonation, softening, GAC filtration, slow sand filtration;
- Waterworks III: canal water, slow sand filtration, GAC, chlorination;
- Waterworks IV: groundwater, aeration, multi layer filtration.

3.2.3 Sampling

The sampling of the waterworks was carried out by the Fraunhofer institute and the ESWE-institute using their own equipment. Details are described in A 2.1 (page 105). The samples were collected in specially cleaned glass bottles and cooled during the transport.
The data on the occurrence of EDCs in waterworks were always confirmed by the analysis of two samples from each sampling site. However, the sampling of the treatment train took place within 3-5 h and was therefore not necessarily proportional to the processing time of the individual treatment steps in the waterworks.

The monitoring results were obtained by using the methods described in Appendix 2.

4 RESULTS

4.1 Evaluation of questionnaires and literature data

Evaluation of the questionnaires returned by the national authorities

Out of the 15 European countries contacted 6 authorities provided information.
Since the discussed environmental chemicals with suspected endocrine activity and the steroid hormones are normally not included in routine measuring programmes only few data were available.
Pesticides are analysed in groundwater monitoring programmes more frequently because of legislative requirements. The reported pesticide concentrations were evaluated for those selected substances which have a potential for endocrine activity.

The information provided by the national authorities included rather reports and references of published results than filled in questionnaires. Due to the overlap of the authority's information and the literature survey the evaluation of the data will be presented together.

Following reports were provided by authorities and EUREAU:

-	Austria	Federal Environmental Agency, Report BE-150: Hormonell wirksame Substanzen in Fließgewässern (Hormonal active substances in surface waters) aggregated data. UBA Austria (1999).
-	Denmark	Monitoring data of groundwater, upper level groundwater and waterworks borings 1993-2000: pesticides and environmental chemicals, aggregated data. Denmark (DK EPA)
-	Germany	Monitoring data of groundwater, pesticides 1996-1999, aggregated data. German Federal Environmental Agency (DE EPA)
-	Ireland	A survey of dangerous substances in surface freshwaters 1999-2000, aggregated data. A. Stephens, Environmental Protection Agency Ireland (IE EPA)
-	Sweden	VA-FORSK Report (VA-FORSK; Research Foundation of the Swedish Water and Wastewater Association). (SE EPA)
-	The Netherlands	LOES Report 2002: Vethaak, A.D., G.B.J. Rijs, S.M. Schrap, H. Ruiter, A. Gerritsen, J. Lahr: Estrogens and xeno-estrogens in the aquatic environment in the Netherlands – Occurrence, Potency and Biological Effects. RIZA/RIKZ-report no. 2002.001.
		KIWA Report: Research on endocrine disruptors and drinking water in the Netherlands, prepared by L.M. Puijker. KOA 01.129, November 2001.

Ghijsen, R.T., W. and Hoogenboezem (2000). Endocrine disrupting compounds in the Rhine and Meuse basin - Occurrence in surface, process and drinking water. Association of River Waterworks – RIWA, Amsterdam, 96 p.

NL RIVM: Pesticides and metabolites in groundwater monitoring network 1993-2000, aggregated data.

- EUREAU — Reports of UK Water Industry Research Limited:

The Implication of Oestrogenic Substances in the Environment. Ref. No 95DW053

Steroid Concentration in the Treated Sewage Effluents and Water Courses. Ref. No 98TX011

Precursors of Halophenols and the related Substances and Fate in Distribution. Ref. No 98TX012

Research Update on Oestrogenic Substances in the Environment. Ref. No 98TX013

Mechanism of Carcinogenicity of Trichlorethene (TCE) and Tetrachlorethene (PCE) and their Metabolites. Ref. No 98TX016

The sorptive Behaviour of Steroid Oestrogens in Sewage Treatment Plants. Ref. No 286_01TX042

Oestrogenic Chemicals and their Behaviour during Sewage Treatment. Ref. No 316_98TX014

Removal of Nonylphenol Ethoxylates by water Treatment Processes. Ref. No 317_98TX015

Effect of Water Treatment Processes on Estrogenic Chemicals. Ref. No 435_96 DW0510

Treatment Chemicals as Sources of Contamination of Drinking Water. Ref. No 436_96 DW0511

- CEFIC — Jülich, W. (2000). Untersuchungen zu endokrin wirksamen Stoffen in den Niederlanden (Investigations on endocrine disrupting chemicals in The Netherlands). Vortrag, 16. Arbeitstagung der IAWR Internationale Arbeitsgemeinschaft der Wasserwerke im Rheineinzugsgebiet (Presentation, 16th Workshop of the IAWR International Working Group of the Waterworks in the Rhine catchment area), 105-124. Stuttgart, 11.-12. Mai 2000, ISBN 90-70671-25-5.

4.1.1 Surface waters

4.1.1.1 Pesticides

In order to get an overview of pesticide concentrations in European surface waters, the database of the COMMPS project was used (COMMPS 1999). The project (Combined Monitoring-based and Modelling-based Priority setting Scheme) was sponsored by the European Union (DG ENV) and the German Federal Environmental Agency (UBA) to establish a list of substances prioritised on the basis of their risk to the aquatic environment and to human health via the aquatic environment in the context of Article 16 of the Water Framework Directive. Monitoring data for organic substances in surface waters were provided from all 15 Member States, but on a varying scale. Reported concentrations were measured between 1994 and 1998. Quality and plausibility of the monitored concentrations were checked as far as possible during the COMMPS project.

Using the CAS numbers of the 22 pesticides and 2 biocides of the questionnaire, a country specific query was performed for the range of concentrations at any monitoring site. The range of the median concentrations and the maximal concentrations at the monitoring sites of each country are summarised in Table 3.
Out of the pesticides in the list of selected substances entries for only 10 pesticides were present in the database: Alachlor, Atrazine, Chlorvenfinvos, Diuron, Endosulfan, Heptachlor, Lindane, Methoxychlor, Simazine and Trifluralin. The data were collected at 2257 monitoring sites in 12 European countries. Lindane, Atrazine and Simazine were most frequently measured, the number of reported data (entries) from 9 countries ranged between 10000 and 146000 per pesticide. Less data were reported for the other pesticides from a lower number of member states (entries were between 36 and 4703).
Highest median concentrations were reported for Simazine, Atrazine and Diuron at 3.4 µg/L, 1.3 µg/L and 1.2 µg/L, respectively. Maximal concentrations of these compounds were measured to be 28.7 µg/L, 14.9 µg/L and 23.1 µg/L, respectively. The median concentrations of the other pesticides were clearly lower. Highest median concentrations were reported for Lindane at 0.17 µg/L, for Chlorfenvinphos at 0.11 µg/L, for Alachlor at 0.07 µg/L, for Trifluralin at 0.04, for Methoxychlor and Heptachlor at 0.01 µg/L. Maximal concentrations of these pesticides could occur at concentrations of 6.25 µg/L (Lindane), 0.22 µg/L (Chlorfenvinphos), 4.12 µg/L (Alachlor), 12.5 µg/L (Trifluralin) and 0.02 µg/L (Heptachlor) and 0.06 µg/L (Methoxychlor).

In answer to the questionnaire Ireland sent concentrations of Atrazine and Simazine in surface waters (Table 4.). Due to the high limits of detection, all samples analysed were negative. In the information from The Netherlands surface water concentrations of Atrazine and Isoproturon in samples above the detection limit were included (Table 5). Maximal concentration of Atrazine

was detected at 0.24 µg/L and of Isoproturon at 0.27 µg/L.
Pesticide concentrations in surface waters monitored in England and Wales 1997 were retrieved from a report of the UK Environmental Agency (2000). The data of the pesticides selected for this project are listed in Table 6. The herbicides Isoproturon and Diuron exceeded 0.1 µg/L most frequently (17.4 % and 11.9 %). Simazine and Atrazine exceeded 0.1 µg/L less frequently, 5.3 % and 4.6 % of the samples analysed, respectively.

Out of the information of the other national authorities, no data regarding the selected pesticides of Table 1 were included.

Based on the results of this survey it can be concluded, that there is inadequate information about the occurrence of some pesticides with suspected endocrine activity in surface waters. The number of pesticides monitored in the different countries varies. Most information is available for Atrazine, Simazine and Diuron. Out of the reported pesticide data, these pesticides most frequently exceeded 0.1 µg/L and can occur in the µg/l range. Based on the COMMPS database, other pesticides with less information (low number of countries, low number of entries) are Alachlor, Chlorvenfinvos, Endosulfane, Heptachlor, Methoxychlor and Trifluralin.

For the other pesticides compiled in Table 1, no data were available in the literature and in the reports of the authorities. Reasons might be that the concentrations were not reported because they were below the detection limit or that they had not been analysed. Since often only data above the limit of detection were reported, no further details can be provided here.

Table 3: Concentrations of pesticides and environmental chemicals in european surface waters between 1994 and 1998, retrieved from the COMMPS database.

substance	country	no of monitoring sites	no of entries	entries > LOD	concentrations at the monitoring sites [µg/L] lowest median*	highest median*	maximal conc.
Alachlor	AU	1	41	1	0.05	0.05	0.22
Alachlor	E	7	19	8	0.0005	0.004	0.012
Alachlor	F	17	170	30	0.0075	0.05	4.12
Alachlor	I	14	69	16	0.025	0.07	0.38
Atrazine	AU	14	311	56	0.015	0.05	1.35
Atrazine	B	51	371	334	0.025	1.315	14.9
Atrazine	D	51	1520	1186	0.005	0.16	3.0
Atrazine	E	7	19	19	0.037	0.2	0.23
Atrazine	F	156	1099	837	0.001	0.805	4.2
Atrazine	GB	382	8075	3459	0.002	0.37	6.4
Atrazine	I	28	166	34	0.015	0.25	1.0
Atrazine	LUX	1	32	10	0.015	0.015	1.2
Atrazine	NL	6	124	122	0.03	0.225	0.8
Chlorfenvinphos	B	5	26	6	0.0025	0.0025	0.177
Chlorfenvinphos	E	5	13	6	0.0005	0.00475	0.009
Chlorfenvinphos	GB	9	125	16	0.002	0.1135	0.222
Diuron	B	31	173	154	0.0425	1.215	23.1
Diuron	D	17	568	174	0.02	0.18	1.5
Diuron	F	32	293	163	0.015	11.3	14.6
Diuron	LUX	1	6	1	0.025	0.025	0.24
Diuron	NL	5	115	81	0.025	0.63	1.40
Endosulfan	GB	9	254	38	0.0005	0.01	0.017
Heptachlor	B	6	56	8	0.002	0.00375	0.011
Heptachlor	D	1	4	1	0.0005	0.0005	0.001
Heptachlor	GR	3	20	3	0.01	0.01	0.020
Lindane	AU	1	1	1	0.02493	0.0249	0.025
Lindane	B	51	378	339	0.0025	0.053	0.285
Lindane	D	42	1451	1044	0.001	0.043	0.160
Lindane	E	10	13	10	0.015	0.166	0.166
Lindane	F	137	974	748	0.001	0.0945	0.380
Lindane	GB	401	11561	6225	0.0005	0.14	6.25
Lindane	GR	5	48	9	0.01	0.01	0.081
Lindane	LUX	1	32	20	0.0065	0.0065	0.027
Lindane	NL	6	147	142	0.003	0.0085	0.12
Methoxychlor	B	2	16	2	0.0025	0.0033	0.015
Methoxychlor	D	1	4	1	0.0005	0.0005	0.001
Methoxychlor	GR	2	16	2	0.01	0.01	0.056
Simazine	AU	5	95	7	0.0075	0.05	0.32
Simazine	B	50	368	324	0.025	3.36	28.7
Simazine	D	40	1403	685	0.004	0.04	0.37
Simazine	E	7	19	19	0.006	0.109	0.248
Simazine	F	86	846	601	0.0075	0.45	2.50
Simazine	GB	383	7711	3106	0.0025	0.65	23.7
Simazine	I	11	117	21	0.015	0.15	0.15
Simazine	LUX	1	32	2	0.025	0.025	0.15
Simazine	NL	6	117	102	0.01	0.14	0.90
Trifluralin	B	17	131	30	0.001	0.0303	0.697
Trifluralin	D	7	265	17	0.0025	0.0415	0.240
Trifluralin	F	8	68	11	0.0075	0.025	0.470
Trifluralin	GB	176	4239	761	0.0005	0.0135	12.5

* for calculation of the median, entries <LOD were considered with half the LOD concentration.

Table 4: Concentrations of Atrazine and Simazine reported from Ireland.

substance	country		concentration [µg/L]	number of samples
Atrazine	IRL,	EPA; 1999-2000	< 0.5,< 0.25	63
Simazine			< 0.5,< 0.25	63

Table 5: Concentrations of Atrazine and Isoproturon reported from The Netherlands.

substance			mean concentration [µg/L]	maximal [µg/L]	number of samples n > LOD*
Atrazine	NL	RIVM, 2000	0.08	0.24	1183
Isoproturon			0.16	0.27	1181

* total number of samples not reported

Table 6: Concentrations of pesticides most frequently exceeding 0.1 µg/L in surface waters (England and Wales).

substance			total number of samples*	% samples > 0.1 µg/L
Atrazine	UK	EA 2000	6409	4.6
Diuron			3759	11.9
Isoproturon			3571	17.4
Simazin			6284	5.3

* pesticides with less than 500 samples and those involved in pollution incident excluded

4.1.1.2 Biocides: Organotin compounds

Due to the extensive use, large amounts of organotin compounds have been introduced mainly into the aquatic environment and significant concentrations of these compounds and their metabolites have been detected. In the environment Tributyltin compounds are expected to exist mainly as Tributyltin Hydroxide, Tributyltin Chloride and Tributyl Carbonate. Concentrations of these substances are generally indicated as TBT cation. As a result of its low water solubility and lipophilic character, TBT sorbs readily onto particles. Recent data on concentrations in surface water have documented a decline in Tributyl levels in the environment, presumably due to the restrictions for the use of antifouling paints on vessels (IPCS 1999). Some examples for TBT concentrations in rivers and creeks are shown in Table 7. Data were retrieved from literature and the reports for Austria, Switzerland, Germany, The Netherlands and United Kingdom.

Highest concentrations of TBT were found in the surroundings of harbours and marinas at concentrations between 0.2 and 0.3 µg/L. In rivers TBT concentrations can range up to 0.05 µg/L. Also in small rivers TBT concentrations up to 0.026 µg/L were reported. Analyses of samples

from the river Elbe in 1991-1993 and a recent survey of rivers in Austria failed to detect TBT above the limits of detection.

TBT can be present in surface water in the range of tens of ng/L. Higher values can occur in the surroundings of marinas. Based on the presented data, a clear decline of the TBT amounts due to the restriction of the use of TBT in antifouling paints can not be observed. Since only few data of 5 European Member States were available, information about a European wide exposure situation can not be provided.

Table 7: TBT concentrations in surface waters

	river		surface water [µg/L]	reference
AU	surface water, n=34	TBT DBT MBT	< 0.01 < 0.01 < 0.01; 1 sample 0.014	UBA Austria 1999
CH	rivers small rivers		< 0.001 – 0.013 < 0.001 - 0.026	Bätscher et al. 1999
CH	rivers		< 0.002 – 0.026	Fent & Hunn 1995
CH	marinas		~ 0.050	Fent & Hunn 1995
CH	Lake Geneva, marina Lake Geneva, background		0.015 – 0.353 0.013	Becker et al. 1992
D	Elbe		n.n. – 0.003	Shawky and Emons 1998
D	small rivers and creeks, Bavaria		< 0.002 – 0.0029	Landesamt Bavaria 1997
D	Bremerhafen, harbour		0.050-0.200	Länge 1996
D	river Weser		0.010 – 0.050	Länge 1996
D	Elbe 1991-93		< LOD	ARGE 1999
D	rivers: Unterelbe, Havel, Lake Constance marina		0.025	Becker and Bringezu 1992
NL	Rotterdam, harbour		0.050 – 0.200	Länge, 1996
UK	surface freshwaters 1997 (England and Wales) n=1861		11 samples (0.6 %) > 0.1 µg/L	UK EA 2000
UK	Wales, Milford Haven		0.01	Länge 1996
UK	coast		< 0.003	Länge 1996

4.1.1.3 Environmental chemicals

4.1.1.3.1 Survey of the COMMPS database

A country specific survey was performed using the data of the COMMPS project, as described in the foregoing chapters. Out of the 19 environmental chemicals selected for the questionnaire, only data for Benzo(a)pyrene and β-HCH were available. The median surface water concentrations of Benzo(a)pyrene at the different monitoring sites in 6 European countries ranged between 0.0008 µg/L and 0.04 µg/L and maximal concentrations were reported at 0.2 µg/L. Median concentrations of β-HCH were reported in the range of 0.0005 µg/L and 0.04 µg/L. Maximal β-HCH concentration was determined to be 0.4 µg/L.

The small output of this data search confirmed that the environmental chemicals with a potential for endocrine activity were not included in monitoring programmes in the European member states between 1994 and 1998. These substances were rather subject to special projects.

Table 8: Concentrations of environmental chemicals in European surface waters between 1994 and 1998, retrieved from the COMMPS database.

					concentrations at monitoring sites [µg/L]		
substance	**country**	**no of monitoring sites**	**no of entries**	**entries > DL**	**lowest median***	**highest median***	**maximal conc.**
Benzo(a)pyrene	AU	11	302	112	0.0015	0.013	0.083
Benzo(a)pyrene	B	9	52	49	0.0027	0.039	0.21
Benzo(a)pyrene	D	17	379	207	0.001	0.017	0.20
Benzo(a)pyrene	DK	2	4	2	0.0105	0.0395	0.074
Benzo(a)pyrene	GB	4	53	40	0.00075	0.006	0.038
Benzo(a)pyrene	NL	4	62	12	0.005	0.0075	0.08
HCH beta	B	24	187	43	0.001	0.012	0.26
HCH beta	D	21	1185	632	0.0005	0.028	0.38
HCH beta	E	1	1	1	0.04	0.04	0.04
HCH beta	F	4	32	8	0.005	0.0135	0.04
HCH beta	GB	37	1506	96	0.0005	0.02	0.09
HCH beta	GR	14	116	22	0.01	0.015	0.401
HCH beta	LUX	1	32	1	0.005	0.005	0.012
HCH beta	NL	7	172	60	0.0005	0.0005	0.007

* median values of different monitoring site were reported. For the calculation of the median, entries <LOD were considered with half of the LOD concentration.

4.1.1.3.2 Bisphenol A

The occurrence of Bisphenol A was reported in rivers from Austria, Belgium, Switzerland, Germany and The Netherlands at concentrations up to 776 ng/L (Table 9). In some samples from Rhine and Meuse very high concentrations up to 1000 ng/L or 21220 ng/L, respectively, were found, but the authors (Ghijsen & Hoogenboezem 2000) expressed some doubts about the latter high concentrations that could not be explained. Therefore, they were not further considered in this report.

It can be seen from the data survey that Bisphenol A can be present in surface waters in the range between tens and hundreds of ng/l. Data could only be collected for surface waters in six countries.

Table 9: Bisphenol A in surface water

	river	Bisphenol A [ng/L]	reference
AU	surface water, n=34, 9 > LOD	< 10 - 75	UBA Austria 1999
B	Meuse, n=29 3 phases: march/june/september 99	< 8.8 - 21220[1] (median 18/39/17)	Ghijsen & Hoogenboezem 2000
CH	Schussen	38	Spengler 2001
D	Elbe, n=10	17 - 776	Heemken et al. 2001
D	Elbe tributaries, n=3	8.9 - 125	Heemken et al. 2001
D	Weisse Elster, n=6	24 - 75	Heemken et al. 2001
D	Havel, Spree, Dahme, n=41	<5 – 410	Fromme et al. 2002
D	Rhine, n=5	42-229	Fromme et al. 2002
D	several rivers and creeks**, n=31, 31 > LOD	0.5 - 14 (median 3.8, mean 4.7)	Kuch & Ballschmitter 2001
D	several rivers	< 0.1 –410 (median 9)	Fromme et al. 2002
D	several rivers	10 - 119	Bätscher et al.1999
D	Elbe, Saale	100	Gies et al. 2001
D	Elbe	17 – 125 (median 57)	ARGE 2000
NL	several rivers, n=97	<8.8 – 1000 [1] (median 45, n=50)	LOES Report 2002
NL	Rhine, n=19 3 phases: march/june/september 99	< 8.8 - 1000[1] (median 93/39/<LOQ)	Ghijsen & Hoogenboezem 2000
NL	surface waters	10 - 160	Belfroid et al. 1999

** rivers: Danube, Nau, Blau, Iller; creeks: Schussen, Laibach, Argen
[1] maximal concentration unrealistic high, opinion of the authors

4.1.1.3.3 Alkylphenols

In Table 10 the concentrations of Nonylphenol and Octylphenol and their respective ethoxylates in surface waters are compiled. The data are from reports provided by authorities and retrieved from literature. Data were available for Austria, Belgium, Germany and The Netherlands.
In general, the Nonylphenol concentrations in surface waters were higher than the Octylphenol concentrations. Highest Nonylphenol concentrations were detected at 4.1 µg/L and 2.72 µg/L in rivers in The Netherlands and Germany. Highest Octylphenol concentrations were 0.27 µg/L and 0.24 µg/L in rivers in Germany and Austria. The maximal value of 6.3 µg/L Octylphenol measured in the LOES project was discussed by the authors to be unrealistic high. The same doubts were expressed from the authors for the maximal values reported for Nonylphenol ethoxylates and Octylphenol ethoxylates. Therefore, these data were not considered in the evaluation. The median concentrations found in the rivers investigated in the LOES project were in a range comparable to the other reported data.
The Alkylphenols were frequently detected in surface waters, but there were also rivers with low concentrations e.g. in the range of 0.001 to 0.05 µg/L Nonylphenol (Elbe and tributaries) or concentrations below the detection limit (Meuse and Rhine).
Nonylphenol monoethoxylates were found up to 3.3 µg/L, while the diethoxylates ranged up to 0.8 µg/L. As a consequence of the lower Octylphenol concentrations compared to Nonylphenol, Octylphenol ethoxylates were found only in trace amounts.

It can be seen from the compiled data, that Nonylphenol and Nonylphenol ethoxylates can frequently be present in surface waters up to hundreds of ng/L. Maximal concentrations can occur at a few µg/L. Octylphenol and Octylphenol ethoxylate concentrations are generally lower, while the concentration of Octylphenol ethoxylates are often in the range of the detection limit. Since data were only available for 4 countries, a conclusion regarding an European wide situation can not be drawn.

Table 10: Alkylphenols and alkylphenol ethoxylates in surface waters

	river	substance	surface water [µg/L]	references
AU	surface water, n=34, 10 > LOD	NP	< 0.03 - 0.571	UBA Austria 1999
B	Meuse 3 phases: march/june/september 99	NP	< (0,16 - 0,71)	Ghijsen & Hoogenboezem 2000
D	Elbe, Havel, Spree	NP	0.01 - 2.72	Fromme et al. 2002
D	several rivers and creeks**, n=31, 31 > LOD	NP	0.0067 - 0.134 (median 0.023, mean 0.032)	Kuch & Ballschmitter 2001
D	Elbe and tributaries, n=13	NP	0.001 - 0.052	Heemken et al. 2001
NL	Rhine 3 phases: march/june/september 99	NP	< (0,15 - 0,58) (median < LOD)	Ghijsen & Hoogenboezem 2000
NL	several rivers[+], n=86, 9 > LOD	NP	< 0.11 - 4.1 (median 0.99)	LOES Report 2002
NL	surface waters	NP	0.14	Belfroid et al. 1999
SP	Anoia and Cardener tributary upstream STP n=2 downstream STP n=4, 3>LOD	NP	 18-51 < 0.15, 42 - 644	Solé et al. 2000
UK	rivers Lae, Dee, Mersey, Thames, Exe n=21, 4>LOD	NP	< 0.2; 0.2-6.2	Blackburn et al. 1999
Uk	Aire n=6, 5>LOD	NP	< 0.2; 3.1-30	Blackburn et al. 1999
AU	surface water, n=34, 12 > LOD	NP1EO	< 0.05 - 0.71	UBA Austria 1999
D	Elbe, Havel, Spree	NP1EO	0.01 - 3.27	Fromme et al. 2002
D	Elbe and tributaries, n=13	NP1EO	0.012 - 0.205	Heemken et al. 2001
AU	surface water, n=34, 3 > LOD	NP2EO	< 0.05 - 0.106	UBA Austria 1999
D	Elbe, Havel, Spree	NP2EO	0.01 - 0.84	Fromme et al. 2002
D	Elbe and tributaries, n=13	NP2EO	0.0036 - 0.084	Heemken et al. 2001
B	Meuse 3 phases: march/june/september 99	NPEO	< 0.11 – 2.45 (median <LOD)	Ghijsen & Hoogenboezem 2000
NL	Rhine 3 phases: march/june/september 99	NPEO	< 0.15 – 2.60 (median < LOD/1.18/<LOD)	Ghijsen & Hoogenboezem 2000
NL	several rivers [+], n=86, 8 > LOD	NPEO	< 0.18 – 87 [1] (median 1.5)	LOES Report 2002
NL	surface waters	NPEO	< LOD	Belfroid et al. 1999a
SP	Anoia and Cardener tributary upstream STP n=2, downstream STP n=4, 2>LOD	NPEO [4+6x]	 < 0.2 < 0.2; 20-100	Solé et al. 2000
SP	Anoia and Cardener tributary upstream STP n=2, downstream STP n=4, 2>LOD	NPEC	 < 0.08 < 0.08; 40-70	Solé et al. 2000

Table 10 continued: Alkylphenols and alkylphenol ethoxylates in surface waters

	river	substance	surface water [µg/L]	references
UK	rivers Lae, Dee, Mersey, Thames, Exe n=21, 2>LOD	NPEO+ NP2EO	< 0.6; 3.2-4.5	Blackburn et al. 1999
UK	Aire n=6, 5>LOD	NPEO+ NP2EO	< 0.2; 6.7-46	Blackburn et al. 1999
AU	surface water, n=34, 1 > LOD	OP	< 0.01 - 0.24	UBA Austria 1999
B	Meuse 3 phases: March/June/September 99	OP	< (0,06 - 0,6)	Ghijsen & Hoogenboezem 2000
D	Elbe, Havel, Spree	OP	0.0004 - 0,27	Fromme et al. 2002
D	several rivers and creeks**, n=31, 31 > LOD	OP	0.0008 - 0.054 (median 0.0038, mean 0.0073)	Kuch & Ballschmitter 2001
D	Elbe and tributaries, n=13	OP	0.0004 - 0.0021	Heemken et al. 2001
NL	several rivers [+], n=86, 8 > LOD	OP	< 0.05 - 6.3 [1] (median 0.3)	LOES Report 2002
NL	Rhine 3 phases: March/June/September 99	OP	< (0,06 - 0,7) (median <LOD)	Ghijsen & Hoogenboezem 2000
NL	surface waters	OP	< LOD	Belfroid et al. 1999a
D	Elbe and tributaries, n=13	OP1EO	0.0006 - 0.0063	Heemken et al. 2001
D	Elbe and tributaries, n=13	OP2EO	0.0006 - 0.0068	Heemken et al. 2001
B	Meuse 3 phases: March/June/September 99	OPEO	< (0,15 - 1,56) (median < LOD)	Ghijsen & Hoogenboezem 2000
NL	Rhine 3 phases: March/June/September 99	OPEO	< (0,14 - 0,68) (median <LOD)	Ghijsen & Hoogenboezem 2000
NL	several rivers [+], n=86, 2 > LOD	OPEO	< 0.16 – 17 [1] (median 11)	LOES Report 2002

** rivers: Danube, Nau, Blau, Iller; creeks: Schussen, Laibach, Argen
[+] rivers: Rhine, Meuse, Ijssel, Scheldt, canal and lakes of northern region
[1] maximal concentration unrealistic high, opinion of the authors
[x] NPEO with an average of four and six ethoxy units

continued

4.1.1.4 Synthetic and natural estrogens

Due to the natural continuous release of estrogens from humans into the sewer system and their incomplete elimination, the hormones enter the aquatic environment in the ng/L range.

Table 11 shows a summary of concentrations of synthetic and natural estrogens in surface waters retrieved from literature and reports. Data were reported for surface waters in Germany, Italy, The Netherlands and the United Kingdom.

The concentrations of the estrogens were partly below the LOD (depending on the methods used and the sample matrix the LOD ranged between < 0.05 and 4 ng/L) or in the lower ng/L range. Maximal 17α-Ethinylestradiol concentrations were detected up to up 5 ng/L. In one study a moderate increase of the amount of the free hormone after enzymatic hydrolysis of the excreted conjugates could be shown.

The natural hormone 17β-Estradiol was detected in surface waters in Germany, Italy, The Netherlands and the United Kingdom up to 12 ng/L. Most of the concentrations reported in literature and in the reports from authorities ranged from < LOD to around 4 ng/L. Maximal values are reported between 5.5 and 12 ng/L.

Estrone, the main metabolite of 17β-Estradiol, was detected at concentrations up to 17 ng/L, whereas the maximal value seems to be very high. Except the latter value, Estrone concentrations in surface waters were between < LOD and 8 ng/L.

Table 11: Synthetic and natural estrogens in surface waters

	river	surface water [ng/L]	references
		17α-Ethinylestradiol	
D	several rivers and creeks**, n=31, 15 > LOD (0.1 ng/L)	0.1 – 5.1 (median 0.4, mean 0.8)	Kuch & Ballschmitter 2001
D	several rivers, lake+, n=79 free hormone 15 > LOQ after hydrolysis 32 > LOQ	 < 0.05 – 2.0 (median <0.05) < 0.05 – 3.0 (median <0.05; 0.1)	Adler et al. 2001
D	several rivers n=15, free hormone	< 0.5	Ternes et al. 1999
D	rivers	< 1 – 3 (median 1)	Wenzel et al. 1998
I	Tiber, n=2, 1 > LOQ	< 0.03; 0.04	Baronti et al. 2000
NL	Rhine and Meuse	< 0,3	Ghijsen & Hoogenboezem 00
NL	surface waters	< 0,3 – 4.3	Belfroid et al. 1999b
NL	surface waters	5	Blok and Wösten 2000
NL	surface water	< 4	Puijker et al. 2001
UK	river Thames, n=6	< 0.2	Xiao et al. 2001

Table 11 continued: Synthetic and natural estrogens in surface water

	river	surface water [ng/L]	references
		17ß-Estradiol	
D	several rivers and creeks**, n=31, 14 > LOD (0.15 ng/L)	0.15 – 3.6 (median 0.3, mean 0.6)	Kuch & Ballschmitter 2001
D	several rivers, lake+, n=79 free hormone 38 > LOQ after hydrolysis 50 > LOQ	 < 0.05 – 2.5 (median <0.05; 0.1) < 0.05 – 3.0 (median <0.05; 0.2)	Adler et al. 2001
D	rivers	< 0.5 – 0.6	Spengler 2001
D	several rivers n=15 free hormone	< 0.5 (median <0.5, 90 percentile 1.0)	Ternes et al. 1999
D	rivers	< 1 – 30 (median < 1)	Wenzel et al. 1998
I	Tiber, n=2, 1 > LOQ	< 0.02; 0.11	Baronti et al. 2000
NL	Rhine and Meuse	< 0,8 - 1	Ghijsen & Hoogenboezem 2000
NL	surface waters	< 0,3 – 5.5	Belfroid et al. 1999b
NL	surface waters	1 – 12	Blok and Wösten 2000
NL	surface water	< 4	Puijker et al. 2001
UK	river Thames, n=6, 4 > LOD	< 0.2 ; 0.5-7.1	Xiao et al. 2001
		Estrone	
D	several rivers and creeks**, n=31, 29 > LOD (0.1 ng/L)	0.1 – 4.1 (median 0.4, mean 0.7)	Kuch & Ballschmitter 2001
D	several rivers, lake+, n=79 free hormone 37 > LOQ after hydrolysis 52 > LOQ	 < 0.05 – 6 (median <0.05; 0.4) < 0.05 – 9 (median 0.3; 0.5)	Adler et al. 2001
D	several rivers n=15, 3 > LOQ free hormone	< 0.5 – 1.6 (median <0.5, 90 percentile 1.0)	Ternes et al. 1999
D	rivers	< 0.5 – 7	Spengler 2001
D	rivers	< 1 – 7 (median < 1)	Wenzel et al. 1998
I	Tiber, n=2, 1 > LOQ	< 0.008; 1.5	Baronti et al. 2000
NL	Rhine and Meuse	< 0,3 – 4	Ghijsen & Hoogenboezem 2000
NL	surface waters	< 0,2 – 5.3	Belfroid et al. 1999b
NL	surface waters	1 – 5.3	Blok and Wösten 2000
NL	surface water	< 4 – 8	Puijker et al. 2001
UK	river Thames, n=6, 6 > LOD	1.7-17	Xiao et al. 2001

** rivers: Danube, Nau, Blau, Iller; creeks: Schussen, Laibach, Argen

+ rivers of south and mid Germany and Lake Constance

4.1.2 Groundwater

4.1.2.1 Pesticides

Monitoring programmes of groundwater for controlling pesticide concentrations are performed in several countries. Data were provided from Austria, Denmark, Germany and The Netherlands. Information on pesticide concentrations in groundwater of the United Kingdom were taken from a report of the UK Environmental Agency (2000). The pesticide concentrations are compiled in Table 12.

Of the 85 pesticides monitored by the UK Environmental Agency in 1997, only 12 exceeded 0.1 µg/L. From these, 12 pesticides which were selected for the questionnaire are listed in Table 12 due to their potential endocrine activity. The pesticide most frequently exceeding 0.1 µg/L was Atrazine (7.4 % of the samples), followed by Diuron and Isoproturon with 2.0 % and 1.5 % of the samples. The frequency of exceeding 0.1 µg/L was 0.4 % for Simazine and Lindane and only 0.2 % for Linuron.

In Austria, monitoring of 237 karst- and fissure groundwater sites is carried out. These groundwater contribute to 50 % to the Austrian drinking water supply. Around 1500 samples were analysed between 1997 and 1999. The pesticides detected above the detection limit were published in the annual report of the Environmental Protection Agency (AU EPA 2000) and evaluated for the pesticides of interest for this study. Desethylatrazine and Atrazine exceeded 0.1 µg/L in 5 and 11 samples, respectively, equal to only 0.3 % and 0.7 % of the samples. Hexachlorobenzene (HCB) was detected in 1 sample at 0.41 µg/L and traces of Simazine of 0.015 µg/L were measured in only 1 sample.

In the monitoring of groundwater intended for drinking water performed in The Netherlands in 2000, Atrazine was found above the detection limit in 270 samples and Simazine in 53 samples, however, the concentrations did not exceed 0.1 µg/L.

The Danish Environmental Protection Agency provided groundwater data of 121 pesticides and metabolites monitored between 1993 and 2000. Out of the pesticides analysed in the groundwater monitoring network 10 of the pesticides compiled in Table 1 were included. From those, Atrazine, Diuron and Isoproturon were detected above the detection limit. However, only Atrazine exceeded 0.1 µg/L in 20 samples equal to 0.4 % of the samples analysed. Maximal Atrazine concentration was 1.52 µg/L. In the upper groundwater 84 pesticides were monitored. Besides the above mentioned pesticides, Simazine was additionally detected above the detection limit. However, the concentrations of Simazine and Diuron were below the drinking water standard of 0.1 µg/L. Atrazine and Isoproturon exceeding 0.1 µg/L were detected in 1 (0.12 %) and 4 (0.65 %) samples, respectively.

In groundwater intended for drinking water 189 pesticides were monitored (waterworks wells). From these, 14 pesticides were selected for the questionnaire (Table 1). The pesticides exceeding 0.1 µg/L were in descending order of their frequencies: Atrazine, Diuron, Simazine, Isoproturon and Linuron but with low frequencies of 0,6 %, 0.13 %, 0.09 %, 0.05 % and 0.03 %, respectively. Maximal concentrations for these pesticides ranged from 0.24 to 1.11 µg/L with highest values for Atrazine.

Out of the 24 pesticides monitored in Germany for concentrations in groundwater between 1996-1999, data for 14 pesticides with suspected endocrine activity could be retrieved. With the exception of Endosulfane, all were detected above the detection limit in a small quantity of samples. The pesticide most frequently exceeding 0.1 µg/L again was Atrazine. Atrazine concentrations ranged from 0.1 to 1.0 µg/L in 2.9 % of the samples, the concentration was analysed above 1 µg/L in 0.07 % of the samples.
Lower frequencies of exceeding 0.1 µg/L were found for Diuron (0.8 %), Simazine (0.45 %), Isoproturon (0.3 %) and 3,4-dichloroaniline (0.2 %). Except 3,4-Dichloroaniline, these pesticides exceeded 1 µg/L in some samples. The other pesticides could only be detected in trace amounts or they were not detected.
In the German groundwater survey also persistent organic pollutants (POPs) were monitored. The concentrations were above the detection limit in some samples, however, they never exceeded 0.1 µg/L (data not shown).

It can be seen from the compiled data, that pesticides with potential endocrine activity can be present in groundwater in frequencies up to 7 %. Atrazine exceeded the limit value for drinking water most frequently, but also for Diuron and Isoproturon maximal concentrations above 0.1 µg/L and 1 µg/L were reported. The reported detection frequencies of the other pesticides with suspected endocrine activity were clearly lower than 1 % or they were not detected, respectively. For the pesticides of Table 1, not mentioned here, no data were available. It should be considered, that pyrethroids are not expected to leach into groundwater because of their low water solubility and high lipophilicity. They are therefore often not included in the monitoring programmes.

The number of pesticides monitored varies between the countries. For this summary data of 4 European Member States had been used. Based on the compiled data it can be assumed, that there are insufficient information regarding other pesticides with potential endocrine activity except the pesticides measured most frequently and mentioned above. (Table 12).

Table 12: Pesticides in groundwater.

year	substance	n	n > LOD	n > 0.1 µg/L	% > 0.1 µg/L		reference
1997	Atrazine	664		49	7.4		UK EA 2000
	Diuron	505		10	2.0		
	Isoproturon	518		8	1.5		
	Lindan	494		2	0.4		
	Linuron	516		1	0.2		
	Simazine	667		3	0.4		
karst- and fissure groundwater					**min [µg/L]**	**max [µg/L]**	
1997-1999	Atrazine	1492	5		0.15	1.10	AU EPA 2000
	Desethylatrazine	1492	11		0.12	0.86	
	HCB	1492	1		-	0.41	
	Simazine	1492	1		-	0.015	
groundwater for drinking water							
2000	Atrazine		270		0.01	0.05	NL RIVM
	Simazine		53		0.04	0.06	
groundwater monitoring network					**median* [µg/L]**	**max [µg/L]**	**reference**
1993-2000	Atrazine	5667	187	20	0.02	1.52	DK EPA
	Diuron	2708	10	0	0.017	0.023	
	Isoproturon	3332	2	0	0.028	0.045	
	Alachlor	293	0				
	Chlorfenvinphos	25	0				
	Endosulfan (alpha)	25	0				
	Endosulfan (beta)	25	0				
	Lindane	25	0				
	Heptachlor	25	0				
	Linuron	1160	0				
upper groundwater							
1993-2000	Atrazine	833	50	1	0.021	0.121	DK EPA
	Diuron	381	2	0	0.013	0.015	
	Isoproturon	618	25	4	0.032	1.07	
	Simazin	820	26	0	0.037	0.05	
	Alachlor	183	0				
	Linuron	221	0				

continued

Table 12 continued: Pesticides in groundwater.

groundwater waterworks wells		n	n > LOD	n > 0.1 µg/L	median* [µg/L]	max [µg/L]	
1993-2000	Alachlor	699	1	-	0.010	0.010	DK EPA
	Atrazin	10639	453	61	0.022	1.114	
	Diuron	3191	20	4	0.020	0.475	
	Isoproturon	5946	34	3	0.019	0.982	
	Linuron	3191	2	1	0.126	0.241	
	Simazin	10577	219	10	0.020	0.420	
	Chlorvenfinvos	34	0				
	Endosulfan (alpha)	52	0				
	Endosulfan (beta)	52	0				
	Lindane	65	0				
	Heptachlor	15	0				
	Methoxychlor	19	0				
	Trifluralin	233	0				
	Vinclozolin	19	0				

groundwater		n	n LOD - 0.1 µg/L	n >0,1-1 µg/L	> 1 µg/L	
1996-1999	Alachlor	3090	2	0	0	DE, UBA
1996-1999	Amitrol	284	3	0	0	
1996-1999	Atrazine	22677	3375	653	16	
1996-1999	Chlorfenvinphos	3899	3	0	0	
1996-1999	3,4-Dichloroaniline	548	2	1	0	
1996-1999	Diuron	11713	136	59	34	
1996, 97, 99	Endosulfan	69	0	0	0	
1996-1999	Heptachlor	1179	8	0	0	
1996-1999	Isoproturon	14713	122	33	5	
1996-1999	Lindane	5890	195	0	0	
1996-1999	Nitrofen	158	11	0	0	
1996-1999	Simazine	22014	900	92	7	
1996-1999	Trifluralin	7081	36	3	0	
1996-1999	Vinclozolin	1848	1	0	0	

* n<LOD considered for the calculation of median

4.1.2.2 Biocides: TBT

Due to the low water solubility of TBT and its sorption properties, it will bind strongly to suspended material such as organic material or inorganic particles. Therefore, transport of TBT applied to soil (via sewage sludge) onto groundwater or migration from rivers into groundwater (via bank filtrate or sediment) is not expected. Nevertheless for worst case scenarios, e.g. sandy soils, uncertainties remain (Bueno et al. 2001).
TBT is generally not included in pesticide monitoring programmes and no data were reported in the questionnaires. Hence, data about TBT concentrations in groundwater could not be retrieved from literature.

4.1.2.3 Environmental chemicals

In the frame of the interdisciplinary Austrian monitoring project ARCEM (Austrian Research Co-operation on Endocrine Modulators) dealing with endocrine modulators in the aquatic environment, among others 59 groundwater samples were analysed on their concentration of alkylphenols and alkylphenol ethoxylates , Bisphenol A and steroids. The project is still ongoing, but it has been reported that alkylphenols, alkylphenol ethoxylates and alkylphenoxy acetic acids were only detected in some cases (personal communication Philipp Hohenblum, EPA Austria).

A summary of the results of the Danish groundwater monitoring performed between 1993 and 2000 is presented in Table 13.

Table 13: Environmental chemicals in groundwater

	substance		median groundwater [µg/L]	max groundwater [µg/L]	reference
groundwater monitoring	Nonylphenol	n=626	0.6	4.2	DK EPA, 1993-2000
	DBP	n=637	1.2	8.1	
	HCH-beta	n=25	< LOD		
upper level groundwater	Nonylphenol	n=34	0.43	0.52	
	DBP	n=26	0.38	0.81	
waterworks boring	Nonylphenol	n=15	< 0.01	< 0.01	

4.1.2.4 Synthetic and natural estrogens

In the environment estrogens should be sorbed to soil particles, due to their elevated lipophilicity (log Kow > 3), and biodegraded in contact with soil (Colucci et al., 2001, Colucci and Topp, 2001). Even after permanent spray irrigation for more than 40 years on agricultural soils

estrogens could not be detected in groundwater (Ternes et al., unpublished data of EU project Poseidon).
Nevertheless, Adler et al. (2001) detected 17α-Ethinylestradiol and Estrone at low concentrations in 57 % and 50 %, respectively, of groundwater samples from South and Middle Germany (n=14). Maximal concentration was 0.6 ng/L for 17α-Ethinylestradiol and 15 ng/L for Estrone. The natural 17β-Estradiol could not be found in the samples. After hydrolyses of the conjugated hormones, 17α-Estradiol could be measured in 1 sample and all samples were positive regarding Estrone.
Hence, the occurrence of synthetic and natural steroid and their conjugated forms in trace amounts in groundwater can not be excluded.

4.1.3 Drinking water

4.1.3.1 Pesticides

According to the Drinking Water Directive pesticides have to be routinely controlled in drinking water and maximal pesticide concentrations are limited to 0.1 µg/L for a single substance and to 0.5 µg/l for the sum of pesticides. Exceptions are regulated in the Drinking Water Directive. Therefore, it can be presumed that concentrations of individual pesticides generally do not exceed this threshold value. The individual measured values are present in the waterworks and respective authorities and not open to the public.

In response to the questionnaire, The Netherlands reported 11 pesticides exceeding the limit of detection in some samples in the year 2000, but only for 3 pesticides concentrations above 0.1 µg/L were measured. However, none of the pesticides with potential endocrine activity listed in the questionnaire was reported at concentrations above the detection limit. In an investigation of Puijker et al. (2001) 0.03 µg/L Atrazine was detected in Dutch drinking water. In the same study, Endosulfane and Lindane were below the limit of detection (0.01 µg/L).

Data on the monitoring of drinking water for pesticides in England and Wales reported by the Drinking Water Inspectorate (DWI) between 1998 and 2001 was provided by John Fawell. The data are compiled in Table 16.

Furthermore, waterworks selected for the case study were asked for the pesticide parameters of their drinking waters. Two waterworks sent the results of pesticide analyses. The data are presented in Table 14 and Table 15. Despite the detection of Atrazine, Simazine, Diuron and Isoproturon in the raw waters, drinking water concentrations of these pesticides and the other analysed pesticides were all below the limit of detection.

Table 14: Waterworks I: General and pesticide data (samples July 02, 2002)

Samples July 2, 2002		**raw water** (reservoir water)	**after ozonation**	**after GAC**	**after chlorination**	**tap water**
laboratory pH		8.4	8.2	7.7	7.9	7.9
on site pH		8.34	8.17	7.68	7.86	7.85
laboratory conductivity	µS/cm	554	555	570	n.d	556
on site conductivity	µS/cm	552	558	567	558	553
total organic carbon	mg/L	5.1	4.1	3.1	2.5	2.4
2,4-D	µg/L	0.05			<0.01	
Atrazine	µg/L	0.066			<0.01	
Diuron	µg/L	0.13			<0.02	
MCPA	µg/L	0.02			<0.01	
Mecoprop	µg/L	0.04			<0.01	
Simazine	µg/L	0.048			<0.01	

The following compounds were investigated but not found (range < 0.01 to < 0.04):

2,4,5-T	Dicamba	Heptachlor epoxide	Parathion
Aldrin	Dieldrin	Hexachlorobenzene	Pentachlorophenol
Bromoxynil	Endosulfan A	Ioxynil	Prometryn
Carbophenothion	Endrin	Isodrin	Propazine
Chlordane	HCH, alpha	Isoproturon	Propyzamide
Chlortoluron	HCH, beta	Linuron	TDE, op
DDE, pp	HCH, delta	Malathion	TDE, pp
DDT, op	HCH, gamma	MCPB	Tecnazene
DDT, pp	Heptachlor	Methoxychlor	Terbutryn

Table 15: Waterworks III: Pesticide data

Samples July 23, 2002		**raw water** (canal water)	**after slow sand filter**	**after GAC**	**after chlorination**	**tap water**
pH		8.32	7.50	7.37	8.01	7.91
NPOC*		2.9	1.5	1.0	1.5	1.6
Atrazine	µg/L	0.16	0.12	< 0.01	0.01	0.01
Simazine	µg/L	-	0.03	< 0.01	< 0.01	< 0.01
Terbuthylazine	µg/L	0.01	0.01	< 0.01	< 0.01	< 0.01
Diuron	µg/L	0.27	0.13	< 0.01	< 0.01	< 0.01
Isoproturon	µg/L	0.01	0.01	< 0.01	< 0.01	< 0.01
Carbendazim	µg/L	0.08	< 0.01	< 0.01	< 0.01	< 0.01
Desethylatrazine (DEA)	µg/L	-	< 0.01	< 0.01	0.01	0.01

The following compounds were investigated but not found (< 0.01):

Desisopropylatrazine,	Cyanazine	Metazachlor	Metobromuron
Chlortoluron	Linuron	Metolachlor	Metoxuron
Chloridazon	Metamitron	Methomyl	Terbutryn

*Non-Purgeable Organic Carbon (equivalent to Total Organic Carbon, TOC)

Table 16: Pesticide parameters in drinking water of the United Kingdom (England and Wales)

year	no of sites investigated	substance	no of samples taken	samples exceeding 0.1 µg/L no	samples exceeding 0.1 µg/L percent	range of exceeding conc. [µg/L]
1997	17	Atrazine	248	26	10	0.1 - 0.26
	10	Diuron	109	19	17	0.11 - 0.27
	8	Isoproturon	161	15	9	0.1 - 0.26
	8	Simazine	82	9	11	0.11 - 0.20
1998	6	Atrazine	85	10	12	0.12 - 0.21
	10	Diuron	193	23	12	0.11 - 0.2
	1	Isoproturon	26	2	8	0.13
	1	Simazine	39	2	5	0.11 - 0.14
1999	6	Atrazine	107	9	8	0.12 - 0.16
	1	Diuron	7	1	14	0.22
	1	Lindane	9	1	11	0.12
	2	Isoproturon	52	2	4	0.16
	1	Simazine	9	1	11	0.3
2000	3	Atrazine	23	3	13	0.12 - 0.13
	4	Isoproturon	100	4	4	0.12
	1	Simazine	12	1	8	0.124
2001	4	Atrazine	60	4	7	0.1 - 0.17
	1	Diuron	25	1	4	0.32
	3	Isoproturon	54	3	6	0.16
	1	Simazine	8	1	13	0.127

As mentioned in the UK EA report (2000), the number of pesticides monitored by water companies varies widely depending on size, geographic location and monitoring strategy. Regarding the pesticides selected for the questionnaire, in England and Wales , all companies monitored the triazine herbicides Atrazine and Simazine. Almost all the companies monitored the herbicides Chlortoluron, Isoproturon, Linuron and Diuron. Fewer measurements were made for organochlorine pesticides (UK EA 2000).

Pesticides which have a potential for endocrine activity can be present in drinking water above the limit value of the drinking water directive of 0.1 µg/l. Comparable to surface and groundwater, most information for drinking water were available for the pesticides Atrazine, Simazine, Diuron, Isoproturon and Lindane. In England and Wales, the frequency of samples exceeding the limit value of the drinking water guideline ranged between 4 and 17 %. In The Netherlands there were no determinations exceeding the standard in 2000.

4.1.3.2 Environmental chemicals

Due to the high requirements on the performance and technical equipment for analysing the low concentrations of EDCs in drinking water, only a limited number of published papers and reports have been available. The data are listed in Table 17. In general, most of the concentrations of the compounds analysed were in the ng/L range or below the limit of detection.

In reports from the Swedish EPA and from Puijker et. al. (2001) concentrations of several phthalates were reported at low concentrations. Highest concentrations were measured for DEHP and DBBP up to 0.13 and 0.15 µg/L, respectively. Information regarding Styrene was only obtained from Ireland. 72 drinking water samples were analysed, but Styrene was below the limit of detection (0.5-1 µg/L).

Data of drinking water were reported in literature and reports from investigations in Belgium, Germany and The Netherlands. In drinking water derived from Rhine and Meuse Bisphenol A was below the detection limit of 8 or 11 ng/L. Though high Bisphenol A concentrations were detected in raw water no contamination of the respective drinking water was found (Table 18). However, the presence of Bisphenol A can not generally be excluded because in another study with drinking water derived from groundwater and surface waters Bisphenol A ranged between 0.5 and 2 ng/L (Table 17).

According to literature and reported data from authorities alkylphenol and alkylphenol ethoxylate concentrations can be present in drinking water in low concentrations at the ng/L range. Nonylphenol concentrations are reported up to 0.29 µg/L and Octylphenol concentrations up to 0.005 µg/L, but most of the samples were negative.
Maximal Nonylphenol ethoxylate content (sum of NP1EO and NP2EO) was found at 4 µg/L and Octylphenol ethoxylates at maximal values of 0.18 µg/L. Despite these positive findings, in the 11 drinking waters derived from Rhine and Meuse Nonylphenol, Octylphenol and OPEO were not detected and NPEO only in three samples at concentrations around 2 µg/L (Table 17). In process waters taken at waterworks near Rhine and Meuse again only NPEO was detected in three samples up to 4.5 µg/L (Table 19).

As mentioned above, only little information was available concerning environmental chemicals with potential endocrine activity in drinking water. For the other chemicals compiled in Table 1 no data were reported. The data for alkylphenols, alkylphenol ethoxylates, Bisphenol and phthalates reveal that these compounds can be present in drinking water in the ng/L range with maximal values in the lower µg/L range for Nonylphenol ethoxylates.
For a sound evaluation of the endocrine potential of drinking water more reliable measurements are needed as well as analyses of further environmental chemicals with potential endocrine activity.

Table 17: Environmental chemicals in drinking water.

	drinking water source	substance	drinking water [µg/L]	reference
S		DEHP	< 0.01 – 0.13	SE EPA 1996
		BBP	< 0.01 – 0.025	SE EPA 1996
		DBP	0.012 – 0.15	SE EPA 1996
		DEP	< 0.1 – 0.057	SE EPA 1996
		DOP	< 0.01, one sample 0.044	SE EPA 1996
		DMP	< 0.001	SE EPA 1996
NL	-	DBP	0,1 - 1	Puijker et al. 2001
	-	DEP	0.1 - <1	Puijker et al. 2001
		PP	< 0.05	Puijker et al. 2001
IE	1999-2000, n=72	Styrene	< 0.5 and < 1	IE, EPA
		Bisphenol A		
D	groundwater / surface water, n=10, 10 > LOD (0.02 ng/L)	BPA	0.0005 – 0.002 (mean 1.1, median 1.1)	Kuch & Ballschmitter 2001
NL	river Rhine, n=11	BPA	< 0.0088 or <0.011*	Ghijsen & Hoogenboezem 2000
B	river Meuse, n=11	BPA	< 0.0088 or <0.011*	* different LOQs depending on the water matrix
		Alkylphenols [µg/L]		
S		NP	< 0.01 – 0.29	SE EPA 1996
B	Meuse, n=11	NP	< (0.18-0.28)	Ghijsen & Hoogenboezem 2000
NL	Rhine, n=11	NP	< (0.18-0.28)	Ghijsen & Hoogenboezem 2000
NL		NP	< 0.01	Puijker et al. 2001
UK	river, n=2	NP	< 0.2	Fawell et al. 2001a
D	groundwater/ surface water, n=10, 10 > LOD	NP	0.003-0.016 (median 0.007, mean 0.008)	Kuch & Ballschmitter 2001
NL	Rhine, n=11	NPEO	< (0.29-0.6); 1 sample 2,02	Ghijsen & Hoogenboezem 2000
B	Meuse, n=11	NPEO	< (0.29-0.6); 2 samples 2,1	Ghijsen & Hoogenboezem 2000
S		NPEO	2.9	Swedish VA-Forsk
S		NPEO	2.9	SE EPA 1996
S		NPEO	4	SE EPA 1996
S		OP	< 0.01	SE EPA 1996
B	Meuse, n=11	OP	< (0.07-0.15)	Ghijsen & Hoogenboezem 2000
NL	Rhine, n=11	OP	< (0.07-0.15)	Ghijsen & Hoogenboezem 2000
NL		OP	< 0.05	Puijker et al. 2001
D	groundwater/ surface water, n=10, 10 > LOD	OP	0.0002-0.005 (median 0.0018, mean 0.002)	Kuch & Ballschmitter 2001
S		OPEO	< 0.01, one sample 0.18	SE EPA 1996
NL	Rhine, n=11	OPEO	< (0.15-0.36)	Ghijsen & Hoogenboezem 2000
B	Meuse, n=11	OPEO	< (0.15-0.36)	Ghijsen & Hoogenboezem 2000

Table 18: Bisphenol A in raw water and corresponding drinking water

Raw water	raw water Bisphenol A [ng/L]	resulting drinking water Bisphenol A [ng/L]	reference
WW Twentekanaal	39 150	< 8.8 < 11	Ghijsen & Hoogenboezem 2000
WW Keizersveer	230	< 11	Ghijsen & Hoogenboezem 2000
WW Brakel	21 220	< 11	Ghijsen & Hoogenboezem 2000

Table 19: Alkylphenols and alkylphenol ethoxylates in process water

	raw water	substance	process water [µg/L]	reference
B	Meuse ;n=8	NP	< (0.16-0.33)	Ghijsen & Hoogenboezem 2000
		NPEO	< 0.17 – 1.5 1 sample > LOD: 1.5	
		OP	< (0.07 – 0.14)	
		OPEO	< (0.16 – 0.34)	
NL	Rhine ;n=15	NP	< (0.18-0.66)	Ghijsen & Hoogenboezem 2000
		NPEO	< 0.28 – 4.5 2 samples >LOD: 1.59 and 4.5	
		OP	< (0.06 – 0.25)	
		OPEO	< 0.15 – 0.70)	

4.1.3.3 Synthetic and natural estrogens

Examples of recent publications of synthetic and natural estrogens in drinking waters are compiled in Table 20. In general, concentrations were below the LODs, which was in the range of 0.05 and 1 ng/L. Published studies were available from Belgium, Germany, The Netherlands and United Kingdom.
While most studies did not detect natural or synthetic estrogens in drinking water, Adler (2001) and Kuch and Ballschmitter (2001) reported natural and synthetic estrogens in drinking water stemming from water supplies and bank filtrate. Maximal concentrations were 0.5 and 1.4 ng/l 17α-Ethinylestradiol, 0.2 and 2.1 ng/ 17β-Estradiol and 0.6 ng/L Estrone. To verify these concentrations in drinking water which were comparable to concentrations found in surface waters, further investigations are need.

Several studies have demonstrated the removal efficiency of the water treatment processes. It could be shown that in drinking water derived from raw water containing Estrone, no estrogen was detected (Table 21). In these studies, out of the natural and synthetic estrogens only Estrone was detected in the raw water.

Concerning the reliable estimation of exposure concentrations, the study of Adler et al (2001) is of importance. They could show that in addition to the free hormones conjugated steroids (steroids bound to glucuronides and sulphates as excreted by humans) contribute to the steroid loads of water samples. The conjugated steroids are enzymatically split in contact with activated sludge (Ternes et al., 1999a) or in the large intestine of humans after ingestion and thus, the active hormones are released.

There are only a few studies dealing with the analysis of steroids in drinking waters from a limited number of countries. For the hormone content in drinking waters the treatment technologies are of major influence. To evaluate the exposure of humans via drinking water in whole Europe, more drinking water data are needed including “realistic worst case” scenario. Prior to monitoring standardization of the methods is of further importance to avoid false positive results and unrealistic high concentrations.

Table 20: Synthetic and natural estrogens in drinking water

	drinking water source	concentration [ng/L]	reference
		17α-Ethinylestradiol	
UK	surface water, n=2	< 0.3	Fawell et al. 2001a
UK	river with significant portion of treated sewage, n=4	< 0.4	Bristol Water Plc 98/TX/01/1 , 1996/97
D	groundwater / surface water, n=10 4 > LOD (0.05 ng/L)	0.15 - 0.5 (mean + median 0.35)	Kuch & Ballschmitter 2001
D	groundwater / surface water, n=28 free hormone 4 > LOQ after hydrolysis 6 > LOQ	 < 0.05 – 1.4 (median <0.05) < 0.05 – 2.4 (median <0.05)	Adler et a. 2001
D	bank filtrate , n=67*	< 1*	Wegener et al. 2001
D	bank filtrate	< 0.5	Ternes 2002
D	n=15	< 1	Stumpf et al. 1996
NL	surface water, n=1	< 0.06	Blok and Wösten 2000
NL	surface water, n=1	<4	Puijker et al. (2001)
NL/B	river (Rhine, Meuse) n=22	< 0.3	Ghijsen & Hoogenboezem 2000
		17β-Estradiol	
UK	surface water, n=2	< 0.3	Fawell et al. 2001a
UK	river with significant portion of treated sewage, n=4	< 0.2	Bristol Water Plc 98/TX/01/1 , 1996/97
D	groundwater / surface water, n=10 5 > LOD (0.1 ng/L)	0.2 – 2.1 (mean 0.7, median 0.3)	Kuch & Ballschmitter 2001
D	groundwater / surface water, n=28 free hormone 4 > LOQ after hydrolysis 12 > LOQ	 < 0.05 – 0.2 (median <0.05; 0.1) < 0.05 – 1.5 (median <0.05)	Adler et a. 2001
D	bank filtrate , n=67*	< 1*	Wegener et al. 2001
D	bank filtrate	< 0.5	Ternes 2002
NL	surface water, n=2	<4	Puijker 2001 (KIWA Report)
NL	surface water, n=1	<4	Puijker et al. (2001)
NL/B	river (Rhine, Meuse) n=22	< 0.3	Ghijsen & Hoogenboezem 2000
		Estrone	
UK	surface water, n=2	< 0.3	Fawell et al. 2001a
UK	river with significant portion of treated sewage, n=4	< 0.2	Bristol Water Plc 98/TX/01/1 , 1996/97
D	groundwater / surface water, n=10 4 > LOD (0.05 ng/L)	0.2 – 0.6 (mean 0.4, median 0.4)	Kuch & Ballschmitter 2001
D	groundwater / surface water, n=28 free hormone 6 > LOQ after hydrolysis 14 > LOQ	 < 0.05 – 0.6 (median <0.05) < 0.05 – 1.2 (median <0.05)	Adler et a. 2001
D	bank filtrate , n=67*	< 1*	Wegener et al. 2001
NL	surface water, n=1	<4	Puijker et al. (2001)
NL/B	river (Rhine, Meuse) n=22	< 0.3	Ghijsen & Hoogenboezem 2000

* concentrations in bank filtrated river water and drinking water

Table 21: Estrone in raw water and corresponding drinking water

Raw water	raw water Estrone [ng/L]	resulting drinking water Estrone [ng/L]	reference
Twentekanaal, Inlet WW	1.6	< 0.3	Ghijsen & Hoogenboezem 2000
Church Wilne Intake	4.1	< 0.3	Fawell et al. 2001a
Stensham Raw (Severn)	1.8	< 0.3	
rivers of south and mid Germany and Lake Constance, n=8; 4 > LOQ	< 0.05 – 2.0 (median <0.05; 0.7)	-	Adler et al. 2001

ww waterworks

4.2 Evaluation of the questionnaires returned by the waterworks

In total, we received 51 filled in questionnaires from waterworks of 5 European countries (Spain, Germany, United Kingdom, Belgium, The Netherlands) with a water production volume up to 287 Mill m^3/year. The greatest number of waterworks participating were from Spain (32) and Germany (16). The other questionnaires were from the waterworks involved in the case study.
The participation on the questioning was on a voluntary basis. Though the participation was favoured by EUREAU, only a relatively small number of questionnaires was returned by the waterworks, mostly private companies.

In total, 17 (33 %) of 51 waterworks reported the occurrence of potential EDCs in their raw water and 12 (24 %) found potential EDCs in their drinking water (Table 15). Excluding the pesticides, the selected EDCs were found in 8 raw waters and 7 drinking waters. The EDCs which were found in the raw water and in drinking water of the participating waterworks are listed in Table 22. Only the database for pesticides and PAHs was adequate. The other EDCs have been analysed by very few waterworks, which makes the interpretation of the results very difficult.

For instance, 5 waterworks analysed Estrone and one found Estrone only in the drinking water, but not in the corresponding raw water. The Estrone contamination of the drinking water was surprising, since the respective waterworks was using GAC filtration and ozonation. Both treatment processes remove estrogens very efficiently. Thus, theoretically only very high concentrations in the raw water could cause a breakthrough of 1 ng/L Estrone. However, due to the low number of samples and the limited information relating to each study, the described result cannot be classified as an outlier. Therefore, further analyses is needed to significantly enlarge the databases.

Theoretically, considering the chemical parameters (log K_{OW}, functional groups) it is also difficult to explain why Nonylphenol appears to pass treatment processes such GAC and ozonation and finally contaminate the drinking water at concentrations of up to 2.1 µg/L (see Table A1/annex). In the same waterworks (WW3 in Tab. A1/annex) 4-tert.-Octyphenol was detected at 0.09 µg/L. Ozonation and GAC filtration should be effective in removing these compounds. Nevertheless, in 4 of 5 waterworks from which data were available Nonylphenol was reported in drinking water. Similar results were found for Bisphenol A, which was detected in 1 of 5 drinking waters at 0.12 µg/L (WW43 in Tab. A1/annex). One possible explanation might be that in these cases a contamination might occur through plastic materials used in the waterworks. However, so far, that assumption has not been confirmed by new data. Similar considerations are true for Phthalates, Nonylphenol ethoxylates, Nonylphenol acetic acids and Styrene. The latter was even found at higher concentrations in drinking water (up to 0.193 µg/L) than in the corresponding raw water (up to 0.07 µg/L).

For all EDCs with a low number of monitored waterworks a final evaluation is not yet possible. However, several reasons should be considered for the contradictions between the theory and the reported analytical data:

a) most of the analytical methods are not standardized (e.g. hormones, alkylphenols),
b) contamination through materials used in the waterworks,
c) incorrect operation of the treatment processes in the waterworks,
d) unexpected influence of other water ingredients on the removal efficiency.

For pesticides the database is much better. However, they are already regulated and the concentrations found in drinking water are relative low (not higher than 0.043 µg/l).

Therefore, it has to be concluded that further analysis of drinking water with standardised methods is essential to confirm whether EDCs are at least sometimes present in drinking water and tap water.

Table 22: Statistical evaluation of the questionnaire: Potential EDCs in raw water and drinking water of waterworks

EDCs detected	**number of waterworks EDCs monitored**	**number of waterworks EDCs found**	**raw water concentrations** [µg/L]	**drinking water concentrations** [µg/L]
Atrazine	51	10	0.01–0.5	< LOQ–0.020
Simazine	51	4	0.01–0.39	< LOQ –0.043
Diuron	51	6	0.0003 - 0.5	< LOQ
Linuron	51	1	0.1	< LOQ
Isoproturon	51	4	0.01 – 2.7	< LOQ
Lindane	51	6	0.0004–0.052	0.0004–0.011
Endosulfan	51	2	0.0003 - 0.013	< LOQ
Benzo(a)pyrene	40	3	0.0001 – 0,05	< LOQ –0.0007
Styrene	6	3	0.01 – 0.07	0.016–0.193
4-Chlorophenol	1	1	0.01–0.05	< LOQ
Tribromophenol	1	1	0.01–0.03	< LOQ
Dimethylphenol	1	1	0.01–0.022	< LOQ
Benzophenone	2	1	0.023–0.054	< LOQ
Diisobutylphthalate	1	1	0.16	0.06
Dibutylphthalate	1	1	0.29	0.1
Benzylbutylphthalate	2	2	0.013–0.84	0.007-0.289
Bis(2-HEPR)phthalate	1	1	0.8–1.3	0.56-0.822
Bis(2-ethylhexyl)phthalate	1	1	1.1–2.9	0.92-1.2
Bisphenol A	5	1	< LOQ – 0.009	< LOQ - 0.12
4-Nonylphenol acetic acid	1	1	0.022	< LOQ
Nonylphenol ethoxylates	1	1	0.18-1.1	0.008-0.11
Nonylphenol	5	5	0.015 - 8	< LOQ – 2.1
Estrone	5	1	< LOQ	0.001
β-Sitosterol	2	2	0.06-0.78	0.01- 0.02

4.3 Results of the case study (Phase II)

4.3.1 X-ray contrast media as tracer for the presence of municipal wastewater

X-ray contrast media are included in the monitoring program, since they are excellent tracer compounds for the presence of municipal wastewater. They are administered up to 200 g/day. However, it has to be noted that they have *no endocrine disrupting properties*. The X-ray contrast media of interest were Diaztrizoate, Iopromide, Iopamidol, Iomeprol, Iohexol, Iothalamic acid and Ioxithalamic acid. The detection was carried out after Solid Phase Extraction (SPE) over ENV+ material with LC-electrospray tandem MS. The limit of quantitation (LOQ) ranged from 10-25 ng/L.

In the raw water of waterworks I and III iodinated contrast media were found at 0.42 µg/L (iopamidol) and 0.21 µg/L (Iopromide), respectively. Furthermore, Diatrizoate, Iomeprol and Ioxithalamic acid were detected. Within the treatment train of waterworks III the concentrations of these compounds were not appreciably reduced and thus, Diatrizoate, Iopromide and Ioxithalamic acid were present at up to 0.45 µg/L after final disinfection and in tap water. Similar results were found for Diatrizoate in waterworks I. Its concentration was mainly constant during the treatment train. The concentrations of the non-ionic iodinated contrast media (Iopromide, iopamidol) decreased with successive treatment stages. Nevertheless, iopamidol and the ionic Diatrizoate were found in drinking water at 0.18 µg/L and 0.10 µg/L, respectively.

It is clear from the literature (Putschew et al., 2000, Ternes & Hirsch, 2000; Ternes et al., 2003) and from the case study that iodinated contrast media are present in treated wastewater, river water and groundwater up to µg/L range, and that they cannot be easily removed with commonly used drinking water treatment processes (e.g. flocculation, bank filtration, ozonation). In particular, the ionic X-ray contrast media Diatrizoate pass unchanged through ozonation and GAC. Only fresh activated carbon can remove Diatrizoate (non published data of EU project Poseidon) but the absorption capacity is rapidly saturated.

Iodinated contrast media can, therefore, be used as excellent tracers for the presence of wastewater. Should EDCs be detected in the absence of iodinated contrast media, other sources than municipal wastewater should be considered.

From the results obtained in the case study the percentage of treated wastewater in the raw water of waterworks I and III was roughly estimated to be 20-30% and 10-20%, respectively. For a better estimation the consumption pattern of X-ray contrast media in the respective country has to be known. Due to these relative high percentage of treated sewage, a significant input of EDCs from municipal wastewater would be expected for waterworks I and III.. This was confirmed by the water supplier.

Iodinated contrast media were not found in the raw water of waterworks II, which leads to the conclusion that the percentage of treated wastewater in the raw water is negligible. Therefore,

EDC contamination *by municipal wastewater* can be largely ruled out. Further sources such as run-off of urine from pastures on which dairy cattle are kept has to be considered. Cows excrete significant quantities of female hormones in their urine. However, it should be noted that in general EDCs can also be emitted by industrial discharges, construction materials, plant protection or by natural sources.

Diatrizoate was found at 0.019 µg/L in the raw water of waterworks IV. Clearly there is an input from the first aquifer layer, which is contaminated by a permanent spray irrigation, into the second aquifer layer. However, the low Diatrizoate concentrations indicated that the percentage of treated wastewater was less than 1 %. Therefore, most of the EDCs (e.g. alkylphenols) present in municipal wastewater should not be present in appreciable quantities.

4.3.2 Chemical analyses

Materials and methods are described in Appendix 2. The detailed results are shown in Table A 20 to Table A 23 in Appendix 4. Table 23 to Table 26 summarise the results of the investigations of the 4 waterworks. Only positive results were shown.

Table 23: Results of the chemical analyses of the water samples of waterworks I (only results > LOQ are given)

Waterworks I		**LOQ**	**raw water (reservoir water)**	**after ozonation**	**after GAC**	**after chlorination**	**tap water**
Industrial chemicals in ng/L							
Bisphenol A		5.0	6.3	n.d.	n.d.	n.d.	n.d.
Phytosteroids in µg/L							
Cholesterol		0.030	0.41	n.d.	n.d.	n.d.	n.d.
Stigmasterol		0.030	0.033	n.d.	n.d.	n.d.	n.d.
Coprostanol		0.030	0.075	n.d.	n.d.	n.d.	n.d.
Campestrol		0.030	0.053	n.d.	n.d.	n.d.	n.d.
Contrast media in µg/L							
Iopamidol		0.025	0.42	0.36	0.28	0.24	0.18
Iopromide		0.010	0.066	0.029	0.014	n.d.	n.d.
Diatrizoate		0.010	0.11	0.12	0.15	0.15	0.10
Organotin compounds in ng/L organotin cation							
Monomethyltin	MMT	0.70	n.d.	n.d.	1.1	n.d.	n.d.
Dimethyltin	DMT	0.90	1.9	1.7	n.d.	n.d.	n.d.
Monobutyltin	MBT	1.0	4.1	2.0	1.3	1.5	1.1
Alkylphenols in ng/L							
4-t-Octylphenol		0.50	4.1	1.2	n.d.	n.d.	n.d.
4-Nonylphenol		6.0	59	27	10.1	n.d.	n.d.
Alkylphenol ethoxylates in ng/L							
4-t-Octylphenol monoethoxylate		0.50	0.9	n.d.	n.d.	n.d.	n.d.
4-Nonylphenol monoethoxylate		2.5	10.0	3.9	n.d.	3.2	2.6
4-Nonylphenol diethoxylate		3.0	12	4.4	n.d.	4.5	n.d.
Alkylphenoxy acetic acids in ng/L							
4-Nonylphenoxy acetic acid		8.0	162	30	n.d.	n.d.	n.d.

LOQ Limit of Quantitation
n.d. below LOQ

Table 24: Results of the chemical analyses of the water samples of waterworks II (only results > LOQ are given)

Waterworks II	**LOQ**	**raw water (seapagewater)**	**before ozonation**	**after ozonation**	**after slow sand filter**	**tap water**
Phytosteroids in µg/L						
Cholesterol	0.030	0.032	n.d.	n.d.	n.d.	n.d.
Steroid hormones in ng/L						
Estrone	0.50	3.0	n.d.	n.d.	n.d.	n.d.
17ß-Estradiol	0.10	0.38	n.d.	n.d.	n.d.	n.d.
Estrogenicity in the YES Screen ng/L		0.06	n.d.	n.d.	n.d.	n.d.
Contrast media in µg/L						
Iopromide	0.010	n.d.	0.014	n.d.	n.d.	n.d.
Organotin compounds in ng/L organotin cation						
Dimethyltin DMT	0.9	n.d.	2.3	2.1	n.d.	n.d.
Alkylphenols in ng/L						
4-t-Octylphenol	0.5	1.6	n.d.	n.d.	n.d.	n.d.
4-Nonylphenol	6.0	21	n.d.	n.d.	n.d.	n.d.
Alkylphenol ethoxylates in ng/L						
4-Nonylphenol monoethoxylate	2.5	12	n.d.	n.d.	n.d.	n.d.
4-Nonylphenol diethoxylate	3.0	7.1	n.d.	n.d.	n.d.	n.d.

LOQ Limit of Quantitation
n.d. below LOQ

Table 25: Results of the chemical analyses of the water samples of waterworks III (only results > LOQ are given)

Waterworks III	LOQ	raw water (canal water)	after slow sand filter	after GAC	after chlorination	tap water
Industrial chemicals in ng/L						
Bisphenol A	5.0	6.1	n.d.	n.d.	n.d.	n.d.
Phytosteroids in µg/L						
Stigmasterol	0.030	n.d.	n.d.	n.d.	n.d.	0.051
β-Sitosterol	0.030	n.d.	n.d.	n.d.	n.d.	0.14
Contrast media in µg/L						
Iopromide	0.010	0.21	0.012	n.d.	0.020	0.029
Diatrizoate	0.010	0.073	0.031	0.041	0.042	0.045
Ioxithalamic acid	0.025	0.037	n.d.	n.d.	n.d.	n.d.
Iomeprol	0.010	0.041	n.d.	n.d.	0.010	0.012
Organotin compounds in ng/L organotin cation						
Dimethyltin DMT	0.9	3.6	2.9	n.d.	n.d.	n.d.
Dibutyltin DBT	1.0	1.6	n.d.	n.d.	n.d.	n.d.
Alkylphenols in ng/L						
4-t-Octylphenol	0.5	1.2	n.d.	n.d.	n.d.	n.d.
4-Nonylphenol	4.0	31	n.d.	n.d.	n.d.	n.d.
Alkylphenol ethoxylates in ng/L						
4-Nonylphenol monoethoxylate	2.5	7.2	n.d.	n.d.	n.d.	n.d.
4-Nonylphenol diethoxylate	1.5	12	n.d.	n.d.	n.d.	n.d.
Alkylphenoxy acetic acids in ng/L						
4-Nonylphenoxy acetic acid	8.0	94	n.d.	n.d.	n.d.	11

LOQ Limit of Quantitation
n.d. below LOQ

Table 26: Results of the chemical analyses of the water samples of waterworks IV (only results > LOQ are given)

Waterworks IV	LOQ	raw water (deep groundwater)	drinking water after aeration	tap water
Contrast media in µg/L				
Diatrizoate	0.010	0.019	0.014	0.018
Alkylphenols in ng/L				
4-t-Octylphenol	0.5	n.d.	0.7	n.d.
4-Nonylphenol	4.0	n.d.	7.9	n.d.

LOQ Limit of Quantitation
n.d. below LOQ

Estrogens

The analytical method (Ternes et al., 1999b) with GC/MS/MS detection was modified, to enable a quantitation within the pg/L range. Instead of 1 L, an enrichment of 10 L water with RP-C_{18} disks (Varian) were used and thus, limits of quantitation (LOQ) down to 0,1 ng/L and limits of detection (LOD) down to 0.050 ng/L were achieved. Natural estrogens were only found in the raw water of waterworks II (mean: 0.38 ng/L 17β-Estradiol, 3.0 ng/L Estrone), where there is probably a direct input from the run-off from pasture with grazing cattle. The most plausible explanation of the natural estrogens found in these circumstances is that they were discharged in the urine of the cows. However, *after coagulation, residence time in a lake (3 months)and rapid sand filtration* the estrogens were not no longer detected in waterworks II. All the other three raw waters did not show any residues of estrogens and contraceptives, although for two waterworks a significant percentage of treated sewage was present and LODs were within the pg/L range (0.050 ng/L for 17β-Estradiol, 0.25 ng/L for Estrone). Hence, all treated waters and all drinking waters investigated did not show any contamination with natural estrogens above the LOD of 0.050 ng/L. The contraceptive 17α-Ethinylestradiol was not found in any raw waters, treated waters and drinking waters above the LOD of 0.050 ng/L.

Summarising the results leads to the conclusion that in the case study with the 4 selected European waterworks the occurrence of natural estrogens and 17α-Ethinylestradiol are not problematic.

If estrogens are present in the raw water, ozonation seems to oxidize them very efficiently (results of the EU project Poseidon/annual report 2002). The second order rate constants of the reaction with ozone for 17α-Ethinylestradiol and the natural estrogens (Huber et al., 2003) as well as results of the ozonation of treated wastewater (Ternes et al., 2003) showed the extremely fast reaction of estrogens with ozone and OH radicals. Additionally, due to their elevated lipophilicity (log $P_{OW} > 3$), estrogens should be efficiently removed by GAC. In the environment and in raw water they should also be sorbed and biodegraded in contact with soil (Colucci et al., 2001, Colucci and Topp, 2001). Even permanent spray irrigation for more than 40 years on agricultural soils did not lead to a groundwater contamination with estrogens (Ternes et al., unpublished data of EU project Poseidon/annual report 2002), after the first lysimeter depth of 40 cm estrogens have never been detected.

Phytosteroids

The phytosteroids are analysed by GC/MS after enrichment over SPE cartridges with 500 mg RP-C_{18} and a silica clean up. The LOQ ranged from 10-30 ng/L and the LOD from 5-10 ng/L. Phytosteroids (Stigmasterol, Campestrol, Coprostanol and Cholesterol) were present in the raw water of waterworks I with a high percentage of treated sewage. After ozonation in waterworks I none of these steroidale compounds with phenolic hydroxy groups were detected. Due to their similarity to the estrogens a quick oxidation by ozone can be expected. Furthermore, Cholesterol was found in the agriculturally influenced raw water of waterworks II. Since Cholesterol is a natural ingredient of many algae and plants, its occurrence is not unexpected. It should be

noted that endocrine disrupting properties for Cholesterol have not been described in the literature. Although no phytosteroids are found in the raw water and in the treatment train of waterworks III, the corresponding tap water contains Stigmasterol (0.051 µg/L) and 17β-Sitosterol (0.14 µg/L). These two phytosteroids could be released into the tap water, a) by leaching out of materials used in the water installation or in the distribution net, (b) by algae, plants growing in the local distribution system. We found that some laboratory products made of natural rubber containing these compounds in appreciable quantities (non published data). Therefore, it cannot be excluded that some sealant or other materials release Stigmasterol and 17β-Sitosterol.

Bisphenol A

The analysis of Bisphenol A was carried out with the analytical method used for the phytosteroids. As surrogate standard Bisphenol F was used. The LOQ was 5 ng/L and the LOD was 2 ng/L. The industrial chemical Bisphenol A was only found in the two raw waters (of waterworks I and III) which have a significant proportion of treated wastewater. However, the concentrations were relative low and ranged between 5 and 7 ng/L. In both waterworks Bisphenol A was removed by ozonation. Similarly to estrogens, ozone oxidises Bisphenol A very efficiently. All of these compounds possess a phenolic group which generally reacts extremely rapidly with ozone. Bisphenol A was not found in treated waters and has not been detected in any drinking waters investigated. Although it is known that Bisphenol A is used as an ingredient of plastics, it was not found in the treatment train and in the tap water.

Organotin compounds

Organotin compounds were analysed by GC/AED after liquid/liquid extraction and a simultaneous derivatisation. The LOQs ranged from 0.70-1.9 ng/L and the LODs from 0.50-1.0 ng/L. In waterworks I Monobutyltin (MBT) was found in the raw water (reservoir water) and in all treated waters including tap water. However, the concentration levels were low, starting with about 4 ng/L organotin cation (OTC) in the raw water and ending with 1.1 ng/L in the tap water. In addition, Dimethyltin (DMT, in the raw water and after the ozonation step) and traces of Mono-Methyltin (MMT, after GAC) were detected. No other organotin compounds were detected or were below the limit of quantitation in waterworks I.

In waterworks II and III Dimethyltin (DMT) and Dibutyltin (DBT) were found in the raw water (canal water). In both cases the compounds were removed during the treatment train. Therefore, in the tap water these compounds were not present. All other organotin compounds were not detected. In waterworks IV none of the organotin compounds analysed were found.

Generally, only very low concentrations of organotin compounds were found in the waterworks. Only those organotins were detected which are used in plastic materials (e.g. as heat and light stabilising agents). Therefore, it seems to be plausible that sometimes plastic pipes or tubing installed in the waterworks may be the sources of the organotin. However, it appeared that the organotin derivative MBT was not appreciably removed by the common treatment processes used in waterworks I (ozonation, flocculation, GAC, slow sand filtration and

chlorination).
In general, organotin compounds with an increasing number of alkyl groups should be more readily sorbed onto GAC or soil, due to their higher lipophilicity (e.g. log K_{ow} of MBT, DBT and TBT are 0.18; 1.89 and 4.70, respectively).

Alkylphenol ethoxylates and alkylphenols

Water samples were analysed for 4-*tert.*-Octylphenol (4tOP) and 4-Nonylphenol (4NP) and the corresponding mono- and diethoxylates. The phenols are degradation products of the 4-Alkylphenol ethoxylates. 4NP belongs to the ubiquitous chemicals in the environment because of the widespread use of Alkylphenol ethoxylates (e.g. 30 % of total use in products for industrial and institutional cleaning, use as emulsifier and stabiliser in plastic materials such as polypropylene and PVC or use as emulsifier in pesticide formulations) (ECJRC 2002, BgVV 2002c). There are, therefore, problems with blanks used in the chemical analysis resulting in relatively high limits of quantitations (LOQ=6.0 ng/L) compared to that of 4tOP (LOQ=0.5 ng/L). The compounds were detected by LC tandem MS after SPE extraction with RP-C_{18} material, a silica clean up and MSTFA silylation.

In waterworks I 4NP was found in the raw water (reservoir water) in concentrations of about 60 ng/L. After ozonation the concentration decreased to about 30 ng/L and after the subsequent GAC filtration to about 10 ng/L. The insufficient removal of the compounds may indicate a low sorption capacity of the charcoal. However, 4NP was not detected after chlorination and in the tap water. 4tOP was detected in 10 fold lower concentrations and was not detected after ozonation. The ethoxylates in the raw water of waterworks I were in the range of 10 ng/L for 4NP1EO and 4NP2EO and about 1 ng/L for 4tOP1EO. 4tOP2EO was not detected. These concentrations were reduced after ozonation to about 4 ng/L (4NP1EO and 4NP2EO) and were below LOQ for 4tOP1EO. GAC filtration seems to remove the compounds completely. However, the samples taken after chlorination showed again trace amounts of the 4NP ethoxylates in concentrations slightly above the LOQ. In the tap water 4NP1EO was still present in levels at the LOQ.

The appearance of the ethoxylates after complete removal in previous water treatment steps may be explained by their possible use in plastic pipes or tubing used in the relevant parts of waterworks I (e.g. after chlorination) This hypothesis would correspond to the finding that Monobutyltin (MBT), which is also typical ingredient of plastic materials, was found in the same samples as well.
However, it should be kept in mind that the concentrations of the alkylphenol ethoxylates found after chlorination and the tap water are very low. Therefore, they cannot be determined precisely and the probability to find concentrations below and above the LOQ is nearly equal.

In waterworks II and III alkylphenols and alkylphenol ethoxylates were found only in the raw water samples. Obviously the water treatment removed the compounds sufficiently and/or no additional source of contamination was present. 4NP was the dominating species, with concen-

trations of about 20 ng/L (WW II) and 30 ng/L (WW III). No ethoxylates of 4tOP were found. The ethoxylates of 4NP were in the range of 5-12 ng/L.

In waterworks IV no alkylphenol and alkylphenol ethoxylate compounds were detected in the tap water samples.

Alkylphenoxy acetic acids

Oxidation of alkylphenol derivatives can result in the formation of alkylphenoxy acetic acids. Therefore, the most prominent species 4-*tert.*-Octylphenoxy acetic acid (4tOP1EC) and 4-Nonylphenoxy acetic acid (4NP1EC) were included into the analytical programme.

The results showed that no 4tOP1EC was found in any sample. In contrast 4NP1EC could be determined in the raw water of waterworks I in high concentrations of about 160 ng/L. Water treatment removed the compound completely (no substance was present after GAC filtration). In the samples of the waterworks II and IV no alkylphenoxy acetic acids were detected. The levels of 4NP1EC in the raw water of waterworks III was in the range of about 90 ng/L. As in waterworks I the substance was removed completely by GAC filtration.

4.3.3 Biological analyses

The material and methods for the in-vitro estrogenic activity are described in Appendix 2.8.

In addition to the chemical analyses an in vitro assay was performed to assess the estrogenic activity of the samples of the waterworks. A bioassay offers an integrated measure of the estrogenic potencies of environmental mixtures without knowing all relevant compounds beforehand. The yeast estrogen screen (YES) assay measures the interaction of a compound with the estrogen receptor. The binding of a compound to the estrogen receptor results in expression of the reporter gene *lac-Z* and finally the enzyme ß-galactosidase is secreted into the medium containing the specific enzyme substrate. The intensity of the formed red product corresponds to the estrogenic activity in the sample and is expressed as relative activity compared to the blank value. Procedural blanks (all components except the sample) were included in each test.
In order to be sure that a result was genuine, relative activity above 10 % of the blank value was considered to be positive.

Due to very low concentrations of the estrogenic substances in the water samples, the water samples were concentrated using solid phase extraction. The concentration of the water sample in the extract is expressed as “water equivalents per solvent [L/mL]” and, accordingly, in the bioassay as “water equivalents per test volume [L/mL]”. The mean values of the relative activities are shown in Figure 1 and the results are included in Table 20 – Table 23 in Apppendix 4.

No estrogenicity could be detected in the water samples, with the exception of raw water at waterworks II. These findings qualitatively confirmed the results of the chemical analyses. The

raw water at waterworks II was the only sample where the most potent EDCs, the natural estrogens 17β-Estradiol (mean: 0.38 ng/L) and Estrone (mean: 3.0 ng/l), were found.

Considering the relative potencies of the estrogens (17β-Estradiol = 1, Estrone = 0.1 (Wenzel et al. 1998, Murk et al. 2002) the chemically analysed estrogen concentration corresponds to around 0.7 ng/L 17β-Estradiol equivalents. In the bioassay, the total estrogenic activity of the sample was determined to be lower at 0.06 ng/L 17β-Estradiol equivalents.

Hence, the bioassay was very sensitive and underscored the positive results of the chemical analysis, where in one raw water sample natural estrogens were found. All other samples, including drinking water and tap water, were negative in the bioassay indicating estrogenic activity.
However, the bioassay detected less 17β-Estradiol equivalents than the chemical analysis. This discrepancy needs to be studied further.

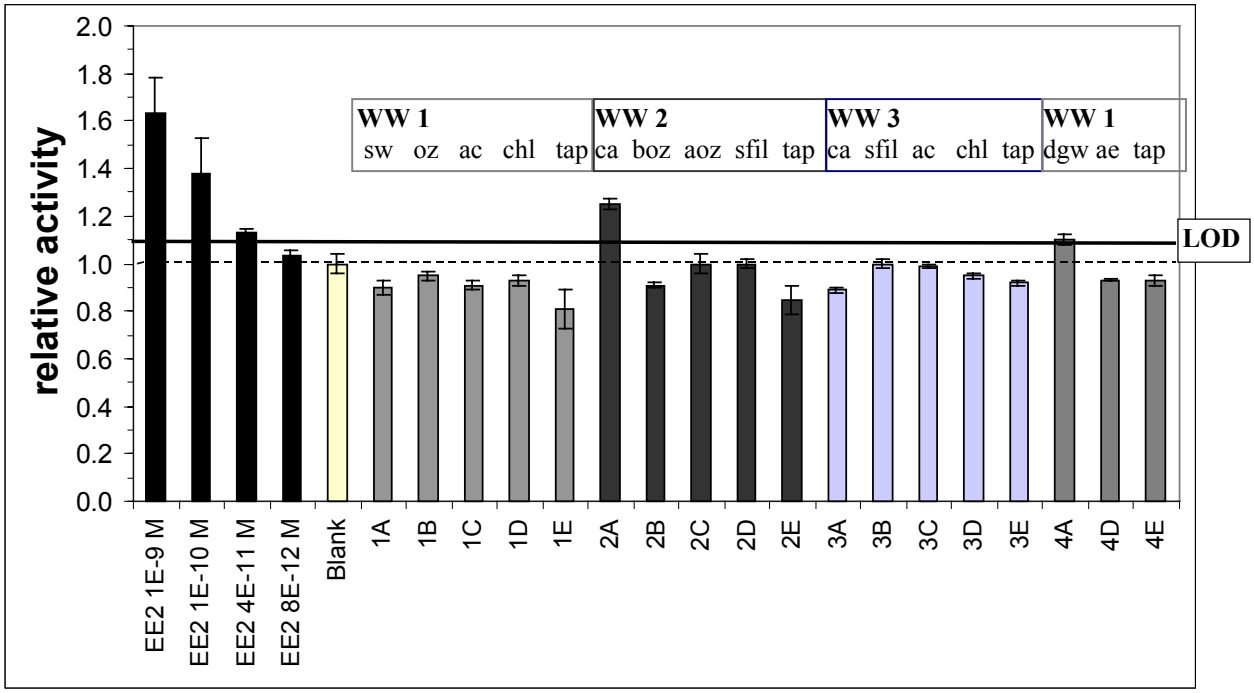

Figure 1: Estrogenic activity of the water samples. Dotted line: blank value; solid line: relative activity above 10 % of the blank value was taken as positive response. (Numbers: Waterworks WW I-IV, A-E: sampling points).

Abbreviations: ac-activated charcoal; ae-aeration; aoz-after ozonation; boz-before ozonation; ca-canal (sw); chl-chlorination; dgw-deep groundwater; oz-ozonation; sfil-sand filtration; sw-surface water; tap-tap water.

5 EVALUATION AND DISCUSSION

In the following chapter the occurrence of the selected substance classes will be summarised and the exposure of humans will be discussed.

Concerning the potential endocrine activity of the substances and their possible effects on humans it was stated in the "Global assessment of state-of-the-science" of the IPCS (2002), that there are currently no data so far that provide firm evidence of direct causal associations between low-level exposure to EDCs and adverse health outcomes in humans. On the other hand, there have been a number of theories proposed for the link between environmental exposure to EDCs and possible endocrine and developmental problems. Comparison and integration of results from independently performed studies with humans is difficult, since many data are collected at different time periods, under different exposure conditions and using different experimental designs. Frequently, exposure data are completely lacking (IPCS 2002).

Many of the outcomes considered have other possible causes and many show a clear familiar link, indicating a genetic component. All of these factors need to be considered in carrying out epidemiological studies. One of the alternative approaches is to consider whether actual exposure occurs to the agents of concern and the extent of such exposure. Of particular concern is the lack of exposure data during critical periods of human development, which are potentially the periods of greatest vulnerability to external influences.

In addition, there is a need to consider the comparative potencies of the various EDCs in relation to the actual exposures. In this regard, the concentrations and potencies of endogenous hormones and phytoestrogens are generally much higher than those of exogenous man-made chemicals. Despite all of these considerations, exposure to EDCs has been suggested to play a role in adverse health outcomes and concerns remain (IPCS 2002).
Potential sources of exposure are through combustion sources (air), contaminants in consumer products, contaminated food and contaminated drinking water.

In general, safety assessment for drinking water is conducted by allocating health-based guidelines for exposure through this medium. For substances intentionally or unintentionally released into the environment such as pesticides or environmental chemicals, ADI or TDI values, respectively, are estimated by an expert group of WHO. Such values have been estimated for substances with threshold mechanism (non-genotoxic mechanisms) after the evaluation of comprehensive long-term animal (including studies on reproduction) and other toxicological studies. The TDI value represents the amount of a substance that – lifelong - can be ingested daily without posing adverse health effects. The values are expressed in mg/kg body weight per day.

For certain compounds analysed in the case study of this project TDI values of the WHO were available, which allowed to benchmark exposure via drinking water against these levels. For the other compounds toxicological knowledge is inadequate to assess effects on human health.

In case the tolerable daily intake (TDI) of a substance included in the case study was available, the maximal possible concentration in drinking water was calculated according to the WHO approach for chemicals with threshold mechanism. The default position taken by WHO is to calculate drinking water guidelines by allowing 10 % of the TDI to come from drinking water and 90 % from other sources. In many cases this is acknowledged to be highly conservative, and for substances for which data exist that identify drinking water as the primary source of exposure a higher proportion of the TDI can be applied to drinking water.

For calculating maximal acceptable permissible drinking water concentrations based on TDI values, the following information and assumptions were used:

adult	60 kg body weight
drinking water intake per day	2 L
contribution of drinking water to TDI	10 %

For some substances no TDI values were available since either adequate data for determining a safe level for humans were lacking or no authority had examined that particular substance at this time. To find a range for a maximum possible drinking water concentration a rough estimation was made based on the WHO approach. This is not a proposal for drinking water standards. The intention for this assessment was to provide information regarding the toxicological relevance of the reported and measured drinking water concentrations. For calculating a TDI, NOAEL of long-term animal experiments or information on effects on humans are needed. The TDI value is derived by applying uncertainty factors to the most sensitive NOAEL. For calculating maximum drinking water concentrations based on a “provisional TDI”, the following information and assumptions were used:

NOAEL	long-term animal experiments, most sensitive endpoint
uncertainty factors	100 for inter and intra-species extrapolation 10 sparse and inadequate data
adult	60 kg body weight
drinking water intake per day, adult	2 L
infant	5 kg body weight
drinking water intake per day, infant	0.75 L
contribution of drinking water to “TDI”	10 %

5.1 Pesticides

5.1.1 Occurrence in surface, ground and drinking water

Pesticides occupy a unique position among the chemicals detected in the environment and in drinking water, since they are deliberately used to control pests in agriculture.

The threshold value for individual pesticides in drinking water is 0.1 µg/L and for the sum of pesticides 0.5 µg/L. Exceptions are possible and regulated in the Drinking Water Directive. The limit value is not only based on toxicological evidence but was set by the European Commission at the limit of detection for organochlorine pesticides, to minimise the occurrence of pesticides in drinking water in accordance to precautionary principles. To provide clean raw water for the preparation of drinking water, the contamination with pesticides should be avoided as far as possible.

The data survey performed in this study revealed that pesticides which have the potential for endocrine activity can be present in surface waters, groundwater and raw water for drinking water at varying frequencies and concentrations.
Regarding **surface waters**, most information is available for Atrazine, Diuron and Simazine from several European Countries. These pesticides most frequently exceeded the drinking water standard of 0.1 µg/L and can partly occur in the µg/L range.
In **groundwater**, Atrazine also exceeded the limit value for drinking water most frequently, but also for Diuron and Isoproturon maximal concentrations above 1 µg/L were reported. The detection frequency of Diuron, Simazine, Isoproturon, 3,4-dichloroaniline, Lindane and Linuron was in general below 1 %. For the other pesticides with suspected endocrine activity selected for the questionnaire no data were available. Pesticides with low water solubility and high lipophilicity, such as pyrethroids, are not expected to leach into groundwater and they are therefore mostly not included in the monitoring. Information were available for Austria, Denmark, Germany, The Netherlands and the United Kingdom. Therefore, a reliable transfer to a union-wide picture regarding groundwater contamination can not be drawn.

In the questionnaires returned from 51 waterworks the frequency of **raw water** samples with pesticides above the detection limit were comparable to the findings mentioned above. Out of the pesticides asked for, the frequencies for the detection of Atrazine, Diuron, Lindane, Simazine, Isoproturon, Endosulfane and Linuron were 20 %, 12 %, 12 %, 8 %, 8 %, 4 % and 1 %, respectively. The raw water concentrations ranged from 0.0005 µg/L to 0.5 µg/L.
Data for raw water were reported from Belgium, Germany, Spain and The Netherlands and the United Kingdom. Therefore, a transfer to a union-wide picture cannot be drawn.

Though the **drinking water** standard is set to 0.1 µg/L for individual pesticides, exceeding concentrations are allowed in exceptional cases. Based on the available data, drinking water samples with concentrations exceeding 0.1 µg/L were reported. In England and Wales, the frequency of samples exceeding the limit value of the Drinking Water Directive ranged between 4 % and 17 % monitored from 1997 to 2001. In the Netherlands no determination exceeded the

standard in 2000. In the questionnaires returned from the waterworks, no exceed of the limit value of 0.1 µg/L was reported. Though in some raw waters pesticides were detected up to 0.5 µg/L, the water treatment was successful in removing the pesticides.
Data for drinking water were reported from Belgium (1 waterworks), Germany, Spain, The Netherlands and United Kingdom. Therefore, a reliable transfer to a union-wide picture cannot be drawn. On the other hand, drinking water has to be monitored for pesticides according to the Drinking Water Directive, and respective data are present at the waterworks but were not reported to us.

Comparable to surface and groundwater, most information for drinking water was available for the pesticides Atrazine, Simazine, Diuron, Isoproturon and Lindane.

Based on the compiled data it can be assumed that there is insufficient information regarding other pesticides with potential endocrine activity, except the pesticides measured most frequently. The number of pesticides monitored can vary widely between the countries and waterworks, because the set of pesticides monitored is based on the likely risk of individual pesticides being present in the water source.

5.1.2 Exposure of humans

As mentioned above, the limit value is not only based on toxicological evidence but was set at the limit of detection, to minimise the occurrence of pesticides in drinking water in accordance to precautionary principles. Health based guidance values are used for an assessment of pesticide concentrations exceeding 0.1 µg/L. For a health based assessment, the WHO approach for deriving ADI values is internationally agreed for substances exhibiting threshold effects. The toxicological basis are long-term studies in animals and the consideration of uncertainty factors (more detail see above). WHO has derived drinking water guidance values (GV) for some 40 pesticides (WHO 1993, 1996, 1998). Depending on the exposure assumptions 1 % or 10 % of the ADI are allocated to drinking water (default value is 10 %). Drinking water guidance values for pesticides are also set by national authorities, such as the German BGVV (Federal Institute for Risk Assessment), and published (BGVV 2001). Guidance values for the pesticides selected for this project are compiled in Table 27.

Table 27: ADI values and drinking water guidance values derived by the German BGVV.

pesticide	ADI (WHO) mg/kg body weight	year of publication	ADI (BGVV) mg/kg body weight	year of publication	drinking water guidance value (GV) (BGVV) µg/L
Amitrol	0.02	1997	0.00003	1991	**0.1**
Chlorfenvinphos	0.00005	1994	0.001	1994	**2**
Diuron			0.007	1997	**25**
Endosulfan	0.006	1998	0.006	1993	**21**
Isoproturon			0.0025	1989	**9**
Lindane	0.001	1997	0.005	1992	**4**
Maneb	0.03	1993	0.03	1995	**105**
Metam-Natrium			0.001	1996	**4**
Pyrethroid: Cypermethrin	0.05	1996	0.05	1989	**175**
Simazin			0.005	1991	**18**
Thiram	0.01	1992	0.01	1993	**35**
Trifluralin			0.0075	1990	**26**
Vinclozolin	0.01	1995	0.005	2000	**18**
Zineb	0.03	1993	0.03	1995	**105**

ADI (WHO) acceptable daily intake derived by WHO
ADI (BGVV) derived by the German BGVV according to WHO approach.
GV guidance value for drinking water, derived by the German BGVV, acceptable drinking water concentration for life-time exposure.

It can be seen that the health-based drinking water guidance-values are higher than the threshold value of the Drinking Water Directive, except for Amitrol, which is equal.

Concerning the potential activity of the pesticides for endocrine activity, the ADI or GV values are assumed to be protective for the consumer, if the assessment was performed using long-term animal studies with enhanced protocols specifically designed to pick up endocrine disrupter effects were used. Due to the increasing knowledge about the effects of pesticides on the endocrine system, it can be assumed that some ADI values will be re-evaluated by the respective committees, if new information is available.

It can be summarised that pesticides which have the potential for endocrine activity can be present in groundwater and raw water for drinking water at varying frequencies and concentrations. Based on the available data, drinking water samples with concentrations exceeding 0.1 µg/L were reported in a few cases. Comparable to surface and groundwater, most information for drinking water was available for the pesticides Atrazine, Simazine, Diuron, Isoproturon and Lindane.
Health based drinking water guidance values are in general higher than the limit value of the drinking water directive. Therefore, direct health hazard via drinking water is not expected. These guidance values are not available for all pesticides.

5.2 Biocides

A data survey on TBT and o-Phenylphenol was performed. No data on o-Phenylphenol in raw water and drinking water were reported. o-Phenylphenol, used as a germicide or fungicide on food, was found in concentrations up to 0.71 µg/L both in influents and effluents of sewage treatment works (Ternes et al. 1998). The Joint FAO/WHO expert committee on food additives (JECFA) allocated an ADI of 0.02 mg/kg body weight for o-Phenylphenol (JEFCA 2001).

5.2.1 Organotin compounds

5.2.1.1 Occurrence

The group of chemicals known as organotin compounds is composed of a large number of compounds with different properties and applications. The most widely used compounds of this group are the di-substituted compounds, which are employed as stabilisers in plastics, including PVC water pipes and the tri-substituted compounds, which are widely used as biocides. Besides the dialkyltins also mono- and octylsubstituted tin compounds are used as stabilisers in plastics. TBT is mainly used as a marine antifouling paint for vessel hulls (> 25 m length). Since 2000, its use for all vessels in inland waters is restricted. Therefore, it was primarily not expected to occur in sewage effluents. But because the stabiliser DBT contains TBT as a technical contaminant at concentrations up to 1 % (based on Sn, German UBA 2000), TBT products can also leach from plastic pipes and reach the aquatic environment. The occurrence of TBT in PVC products was confirmed by a Danish study detecting TBT in PVC consumer products such as vinyl floor, gloves, vinyl wall paper, up to 2.5 mg/kg (Galten and Fuhlendorff 2001). In the environment, TBT sorbs readily onto particles as a result of its low water solubility and high lipophilicity. Therefore, transport of TBT through soil to groundwater is unlikely.

In the literature TBT concentrations in **surface waters** were reported up to 0.05 µg/L. Higher values were reported from the vicinity of marinas ranging from 0.2 to 0.3 µg/L. No groundwater data could be retrieved from literature and no information about TBT in surface, groundwater and drinking water was given in the questionnaires returned from authorities. In the questionnaires returned from 51 waterworks, no data about TBT were reported. TBT was not included in the monitoring of the raw waters and drinking waters.

In the case study, the occurrence of several organotin compounds in **raw water, process water and drinking water** at four waterworks was investigated. In raw water at three waterworks only MMT, DMT and MBT were detected at low concentrations ranging between 0.0016 µg/L to 0.0041 µg/L. The detected compounds are typical for stabilisers in plastic material.
In the **tap water samples** no organotin compounds could be detected with the exception of 1 waterworks. In the respective tap water sample only MBT was measured at a very low concentration (0.0011 µg/L) near the limit of quantification of 0.001 µg/L. TBT was not detected in any sample.

5.2.1.2 Human exposure

From the group of organotin compounds TBT is of most concern, since it is a strong aromatase inhibitor, responsible for endocrine disrupting activity in molluscs by prolonging the half-life of testosterone in the organism; it is of higher immunotoxicity than the other organotin compounds.

It is generally agreed that main exposure of humans to TBT occurs via seafood. To a negligible extent, exposure may occur via skin from biocides in clothing and from PVC applications on clothing. Most TBT data are known about concentrations in fish and seafood, e.g. levels in herring from the North Sea can reach 6.4 µg TBT/kg fresh weight (BGVV 2000).

The risk assessment and the estimation of safe food levels for TBT is based on the most sensitive long-term animal study with rats. The most sensitive parameter was immunotoxicity with a NOAEL of 0.025 mg/kg bodyweight (WHO 1990). Based on this toxicity endpoint, the WHO estimated for TBTO a TDI of 0.0003 mg/kg body weight per day (IPCS 1999). Because of structural similarities, this TDI can also be applied for TBT chloride (BGVV 2000). As stated in the BGVV opinion (2000), an inhibition of the aromatase in vivo can not be excluded. But it is assumed that the dose for the enzyme inhibition is relatively high. As a consequence, other more sensitive parameters will be predominant rather than respective effects on the sexual hormone system. This aspect will be proven further on. Though TBT is the most active substance of the group of organotin compounds it should be considered, that for some other compounds of this group the toxicological knowledge is limited. An additive effect of compounds with similar mechanism is normally regarded as the most appropriate assumption and a comparable mechanistic profile regarding immunotoxic effects in subchronic- and chronic feeding studies with rats was observed for TBT, DBT, DOT and TPT (BGVV 2000). These effects are not associated with aromatase inhibition and therefore, are unlikely to be associated with endocrine disruption.

The guideline value of the WHO Guideline (1999) for drinking water derived from the TDI value was set to 2 µg/L TBT, based on 20 % of the TDI allocated to drinking water.

Regarding the drinking water concentrations of this study, following **worst case assessment for human exposure** towards TBT was made: the LOQ of 1.6 ng/L was used because TBT was not detected. This results in an exposure of 0.0032 µg TBT per day or 0.000053 µg/kg body weight per day for an adult. An infant would be exposed to 0.0012 ng TBT per day equal to 0.00024 ng TBT/kg body weight per day.
The calculated drinking water concentration is around lower by a factor of 1000 compared to the WHO guideline value and contributes to 0.02 % to the tolerable daily intake.
It can therefore be concluded, that exposure of humans via drinking water is of minor importance.

Table 28: Exposure levels of TBT

	concentration in drinking water		exposure via drinking water			
	µg/L TBT	% of guideline value	daily intake (2 L) µg/day *	daily exposure level µg/kg bw/day	% of TDI (0.25 µg/kg bw/day)	reference
WHO drinking water guideline value* based on the TDI.	2	100%	4 (adult)	0.067 (adult)	~ 20 %	WHO 1999
maximal TBT concentration in dw, assumed **	0.0016 **(LOQ)**	0.08%	0.0032 (adult) 0.0012 (infant)	0.000053 (adult) 0.00024 (infant)	0.02 %	this project

* 20 % contribution of drinking water to the TDI according to WHO (1999), 2 L dw intake, 60 kg adult (rounded values).
** LOQ of the chemical analyses was used, due to negative results in the case study. No literature data available.

In summary:

Out of the organotin compounds TBT is of most concern. TBT was detected in surface waters up to 0.05 µg/L. No data were available regarding organotin compounds and especially TBT in groundwater and drinking water. In the case study, trace amounts (ng/L) of MBT, DBT and DMT were found in raw water. These compounds are typically used as stabilisers in plastics. TBT was not detected in final drinking water and tap water of the case study. However, this should be verified at a European level.

Due to its physico-chemical properties TBT sorbs to particles and should generally be removed by passage through soil or sediment to groundwater and by the respective water treatment. Nevertheless, leaching from plastic products, such as pipes, can result in TBT concentrations in the ng/L range.

Based on the worst case assessment for human exposure towards TBT it seems to be unlikely, that drinking water can significantly contribute to the organotin exposure. Therefore, direct health hazard via drinking water is not expected.

5.3 Environmental chemicals

Information about exposure of humans was collected for the environmental chemicals selected for chemical analyses in the case study.

5.3.1 Bisphenol A

5.3.1.1 Occurrence

Bisphenol A is used for the production of epoxy resins and polycarbonates, as an antioxidant in PVC plastics and as an inhibitor of end polymerisation in PVC. Polycarbonates are used in food contact materials and epoxy resins are used in protective linings for food and beverage cans and vats.

Concentrations of Bisphenol A in **surface waters** were reported in literature and from authorities in rivers from Austria, Belgium, Switzerland, Germany and The Netherlands up to 0.8 µg/L. For a union wide picture data from the other European countries are required.

Data on Bisphenol A in **groundwater** were completely lacking. In Austria, a monitoring of groundwater is currently being performed including determinations of Bisphenol A. Results are not yet available (ARCEM Austrian Research Co-operation on Endocrine Modulators).
In the questionnaires returned from the waterworks 5 out of 51 waterworks reported determinations of Bisphenol A in raw waters. Concentrations above the limit of detection were reported from 2 waterworks ranging between 0.0025 µg/L and 0.009 µg/L.
In the case study Bisphenol A was found **in raw water** at 2 of the 4 waterworks at around 0.006 µg/L, a concentration close to the limit of quantification of 0.005 µg/L.

Concentrations of Bisphenol A in **drinking water**, if detected, were in the lower ng/L range. Data from the literature reveal that Bisphenol A can occur in drinking water at concentrations between 0.0005-0.002 µg/L. In the questionnaires returned from the waterworks drinking water concentrations at 0.12 µg/L Bisphenol were reported from one waterworks. One possible explanation for this concentration, which is higher than the corresponding raw water concentration, might be a contamination through plastic materials used in the waterworks. However, so far, this assumption has not been confirmed by new data.

In the drinking water samples of the four waterworks analysed in the case study, no Bisphenol A could be detected.

5.3.1.2 Human exposure

The main route of human exposure is food in contact with plastics (SCF 2002a, BGVV 2002). The European Scientific Committee of Food (SCF) assumes in a realistic worst case estimate a daily intake of Bisphenol A with canned food of 0.37 µg/kg body weight for adults, 1.2 µg/kg

body weight for a 4-6 year old child and 1.65 µg/kg body weight for infants. The intake of infants concerns also formula stored in plastic bottles (Table 29).

The estrogenic potency of Bisphenol A in in-vitro assays is lower by a factors of around 100 000 compared to the natural hormone 17β-Estradiol. Based on the evaluation of toxicological animal studies with enhanced protocols specifically designed to pick up endocrine disrupter effects the SCF estimated for humans a tolerable daily intake (TDI) of 0.01 mg Bisphenol A per kg body weight per day.

The results of the study reveal that concentrations of Bisphenol A in drinking water, if detected, are in the lower ng/L range. The maximal drinking water concentration at 0.12 µg/L reported from 1 waterworks seems to be very high, compared to the other reported and measured data, however, in the absence of confirmation, this value will be used for a worst-case exposure estimation. The other analytical data indicate that this could be expected to be an extreme worst case.

Estimating a worst-case exposure using the maximal reported concentration of 0.12 µg/L and 2 L drinking water consumption per day, a daily intake of 0.24 µg can be calculated for an adult, corresponding to an exposure level of 0.004 µg/kg body weight per day. The exposure of an infant would be higher by a factor of 4.5, due to the higher ingestion of drinking water relative to the body weight. An 5 kg infant would be exposed via drinking water to 0.09 µg Bisphenol A per day or 0.018 µg/kg body weight per day, respectively (Table 29).
According to the WHO approach, 10 % of the tolerable daily intake of 0.01 mg/kg body weight is attributed to an intake of 2 L drinking water. These assumptions allow a maximal Bisphenol A concentration in drinking water of 30 µg/L, orders of magnitude higher than the so far measured concentrations. **The measured Bisphenol A concentrations were maximal 0.4 % of the calculated "provisional drinking water guidance value"**

Even based on the above described worst case assumption, the contribution of drinking water to the human exposure to Bisphenol A is lower than the contribution of canned food or formula stored in polycarbonate bottles. **For infants, the estimated worst case Bisphenol A exposure via drinking water is around 0.2 % of the temporary TDI value estimated by the SCF.**

Based on the current assessment concerning effects on humans, the Bisphenol A quantity consumed via drinking seems to be unlikely to pose an adverse health threat to humans. However, as also stated in the comment of the SCF, the limit values have to be revised, if new data of current studies are published giving new insight in effects of Bisphenol A. For example, Schönfelder et al. (2002) investigated for the first time the internal exposure of consumers to Bisphenol A and there are hints for a prenatal exposure of humans. However, the study provides no indications on adverse effects of children.

In summary:

Bisphenol A can be present in surface waters in the upper ng/L range. Groundwater data are lacking so far. Bisphenol A was reported to be present in raw water and drinking water in a few cases in the lower ng/L range. In the tap water samples of the case study Bisphenol A could not be detected. Some reported controversial results need further verification.

It can be concluded from the existing data, that drinking water contributes to a minor part to exposure of humans to Bisphenol A. Based on the current assessment concerning effects on humans, the Bisphenol A quantity consumed via drinking seems to be unlikely to pose an adverse health threat to humans.

Due to the limited information on Bisphenol A concentrations in raw water and drinking water, the database should be enhanced to enable an European wide picture.

Table 29: Exposure levels of Bisphenol A

	concentration in drinking water		**exposure via drinking water**			
	µg/L	**% of guidance value**	**daily intake (2 L) µg/day ***	**daily exposure level µg/kg bw/day**	**% of TDI (10 µg/kg bw/day)**	**reference**
drinking water, provisional standard* based on the TDI.	30	100 %	60 (adult)	1 (adult)	10 %	this project
Bisphenol A concentration, worst case assumption, reported for drinking water	0.12	0.4 %	0.24 (adult) 0.09 (infant)	0.004 (adult) 0.018 (infant)	0.04% (adult) 0.2 % (infant)	this project
			exposure via food			
	concentration		**daily intake µg/day**	**daily exposure level µg/kg bw/day**		
Bisphenol A in canned food, realistic worst case				0.37 (adult) 1.65 (infant)	3.7 % 16.5 %	SCF 2002a

*10 % contribution of drinking water to the TDI, 2 L daily intake, 60 kg adult

5.3.2 Alkylphenols

5.3.2.1 Occurrence

Nonylphenol and Octylphenol are degradation products of the respective polyethoxylates used as surfactants in household and industrial cleaners (30 % of total use). The cleaners mainly contained Nonylphenol polyethoxylates. In Europe, all major manufacturers of detergents have agreed a voluntary ban on the use of Nonylphenol ethoxylates in domestic detergents. PARCOM Recommendation 92/8 requires Member States to achieve the phase out of Nonylphenol ethoxylates in domestic detergents by 1995 and in all detergent applications by 2000 (EU RAR 2002).
Nonylphenol ethoxylates are also used as emulsifier and stabiliser in polypropylene and PVC. Nonylphenol derivatives are added to pesticide formulations as an emulsifier. Further uses are in emulsion polymerisation, as a textile auxiliary, captive use by the chemical industry as a leather auxiliary, agricultural use, use in paints and other niche market uses (EU RAR 2002, BgVV 2002c).

Nonylphenol and Octylphenol were frequently detected in **surface waters**. In literature and in reports from authorities Nonylphenol concentrations were reported up to 644 µg/L. Data were available from Austria, Belgium, Germany, Spain, The Netherlands and United Kingdom. The extreme high Nonylphenol concentration (644 µg/L) was found downstream of a sewage treatment plant and it can be assumed that this value does not represent the common river concentration. Upstream of the sewage treatment plant maximal 52 µg/L were detected. This value and the concentration of 30 µg/L measured in the river Aire were the maximal values of all reported data.
According to the use of the surfactant classes, Octylphenol occurs in the environment at lower concentrations compared to Nonylphenol. The highest Octylphenol concentration reported was 0.27 µg/L. Besides positive findings, concentrations below the limit of detection were also reported.
Nonylphenol monoethoxylate were found up to 3.3 µg/L while the diethoxylates ranged up to 0.8 µg/L. As a consequence of the lower Octylphenol concentrations, Octylphenol ethoxylates were found only in trace amounts and below the limit of detection.
Based on the presented data, a wide variation of Nonylphenol concentrations in the European Member States can be assumed, due to differences in the purification capacity of the sewage treatment plants discharging into the river.

For **groundwater** only little information was available. In the Danish groundwater monitoring Nonylphenol was measured up to 4.2 g/L. In waterworks borings Nonylphenol was not detected (LOD 0.01 µg/L). In samples taken from process water Alkylphenols and Alkylphenol ethoxylates could not be detected except in one sample, which contained 4.5 µg/L NPEO. In the frame of the interdisciplinary Austrian monitoring project ARCEM (Austrian Research Cooperation on Endocrine Modulators) dealing with endocrine modulators in the aquatic environment, among others 59 groundwater samples were analysed on their concentration of

alkylphenols and alkylphenol ethoxylates. The project is still ongoing, but it has been reported that alkylphenols, alkylphenol ethoxylates and alkylphenoxy acetic acids were only detected in some cases (personal communication Philipp Hohenblum, EPA Austria).

Out of the 51 waterworks responding to the questionnaire 5 analysed Nonylphenol in **raw water** samples, and all 5 waterworks detected Nonylphenol ranging from 0.015 to 8 µg/L. Nonylphenol ethoxylates, Nonylphenol acetic acid and Octylphenol were measured by only one waterworks each and the concentrations were up to 1.1 µg/l NPEO, 0.02 µg/L NPEC, and 0.2-1.4 µg/L OP.
In the case study, raw water samples at 3 out of 4 waterworks contained alkylphenolic compounds in the ng/L range. Nonylphenol ranged from 0.02 µg/L to 0.06 µg/L, Octylphenol from 0.0012 µg/L to 0.004 µg/L. Nonylphenol mono- and diethoxylates concentrations were between 0.007 and 0.012 µg/L. OP1EO was only present in raw water at 1 waterworks in trace amounts.

According to literature and reported data from authorities alkylphenols and alkylphenol ethoxylate concentrations can be present in **drinking water** in low concentrations at the ng/L range. Nonylphenol concentrations were reported up to 0.29 µg/L and Octylphenol concentrations up to 0.005 µg/L, however, most of the samples were negative. The maximal Nonylphenol ethoxylate content was found at 4 µg/L, and the maximal value for Octylphenol ethoxylates was 0.18 µg/L.

Out of the data of the questionnaires returned from the waterworks Octylphenol was detected in one drinking water sample at 0.09 µg/L, and also Nonylphenol was reported in drinking water in 4 waterworks up to 2.1 µg/L. These concentrations are astonishing because the water treatment technology of these waterworks should be effective in removing these compounds.
In the **tap water samples of the case study**, Nonylphenol and Octylphenol were not detected. Out of the other compounds NP1EO was measured at a concentration near the limit of detection (0.008 µg/L) in 1 waterworks and NPEC was detected in trace amounts in another waterworks (0.011 µg/L).

5.3.2.2 Human exposure

Food is expected to be the main route of exposure of humans under normal conditions. Günther et al. (2002) analysed various food stuffs, except drinking water. Based on a survey of the German market basket, the authors calculated a daily intake of 7.7 µg Nonylphenol for an adult and 1.4 µg Nonylphenol for an infant fed with infant formulas. A worst case estimate by the Scientific Committee on Food calculated that the daily exposure for an adult via food was between 0.48 – 1.6 µg/kg NP body weight per day (SCF 2002b). In the Risk Assessment Report (EU RAR 2002) the theoretical combined exposure is summarised as follows: “The highest exposure of an individual would occur if they apply speciality paints (2 mg/kg/day), use a pesticide product (0.35 µg/kg/day), cosmetics (0.2 µg/kg/day) and are exposed via food packaging materials (0.2 µg/kg/day) whole living in the locality of a textile factory (4.42 mg/kg/day). The

total exposure would be approximately 6.4 mg/kg/day." The data regarding human exposure via food are compiled in Table 30.

Table 30: Exposure of humans with Nonylphenol via food

	daily intake µg/day	**daily exposure level µg/kg bw/day**	**reference**
Germany (mixed diet) adult	7.5	0.13	Günther et al. (2002)
infants fed with infant formulas	1.4	0.28	
baby breastfed	0.2		
via food packaging materials		0.2	EU JRC 2002
worst case assumption, mixed food		0.48 – 1.6	SCF 2002b

For food consumption no TDI values of the WHO for Nonylphenol and Octylphenol and the respective ethoxylates are available (BGVV 2002c). Nonylphenol is listed in the Synoptic Document in the SCF list 8 (substances for which only scanty and inadequate data were available) (SCF 2002b).
Although there is clear evidence for the estrogenic activity of Nonylphenol and Octylphenol in whole animals, particularly during development, it is difficult to use the available data to determine a safe level for humans with any confidence. However, the level of estrogenic activity is considerably lower than natural hormones (Fawell and Chipman 2001, Müller et al. 1998). Compared to the natural hormone 17β-Estradiol the estrogenic potency in in-vitro assays was found to be 10 000 – 100 000 times lower (Murk et al. 2002).

Based on the data evaluation of Nonylphenol for the toxicological assessment of Nonylphenol in the frame of the EU Commission Regulation on Risk Assessment of Existing Substances the most sensitive parameters were sperm count, ovary weight and oestrus cycle in rats. The NOAEL was estimated to be 15 mg/kg bw (EU RAR 2002).
In order to estimate a range for a provisional maximal possible drinking water concentration, the NOAEL of the rat study was transformed according to the uncertainty factor model of the WHO using an uncertainty factor of 1000: a provisional TDI can be calculated with 0.015 mg/kg body weight and the derived provisional drinking water guidance value is 45 µg/L Nonylphenol.
In the toxicological evaluation of the Danish Institute of Safety and Toxicology a higher uncertainty factor of 3000 was used and a provisional TDI value of 0.005 mg/kg body weight was derived (Nielsen et al. 2000). Using this TDI value, a provisional drinking water guidance value of 15 µg/L can be calculated (Table 31)

All concentrations of the single compounds and the sum of analysed alkylphenols were significantly lower than the above rough estimation of acceptable drinking water concentration derived from toxicity data.

The exposure levels presented and discussed above are summarised in Table 31. The Nonylphenol exposure of humans were calculated with the maximum concentrations reported in the literature and in the questionnaires. The measured drinking water concentrations of the case study were below the limit of quantification (0.006 µg/L).
The derived exposure levels for humans via drinking water are between 0.01 and 0.07 µg NP/kg body weight. Compared to the Nonylphenol intake of an adult via mixed food the worst case intake using drinking water with 2.1 µg/L Nonylphenol is lower.
Compared to the "provisional guideline value for drinking water" derived by the Danish EPA Nonylphenol intake via drinking water is 2 % - 14 % of this value and contributes between 0.07 % and 0.5 % to the provisional TDI.

Table 31: Exposure levels of Nonylphenol: estimated and measured

	concentration	**daily intake µg/d NP***	**daily exposure level µg/kg NP bw/day**	**% of "TDI" (15 µg/kg bw/day)**	**reference**
		via food	**via food**		
mixed food, adult		7.5	0.13	0.9 %	Günther et al. 2001
mixed food, adult, worst case scenario			0.48 – 1.6	3.2 - 10.7 %	SCF 2002 b
infant formulas, infants		1.4	0.28	1.9 %	Günther et al. 2001
	µg/L (% of provisional standard)	**via drinking water (2 L)**	**via drinking water**		
„drinking water, provisional standard"** based on a NOAEL (rat) of 15 mg/kg bw.	45	90	1.5	10 %	this project EU RAR 2002
„drinking water, provisional standard"** based on a NOAEL (rat) of 15 mg/kg bw and higher uncertainty factor.	15 (100 %)	30	5		Nielson et al. 2000 (Danish EPA)
drinking water, maximal concentration, literature survey	0.3 (2 %)	0.6	0.01	0.07 %	Swedish VA Forsk
drinking water worst case, maximal concentration, questionnaire	2.1 (14 %)	4.2	0.07	0.5 %	this project

* drinking water: 2 L drinking water intake per day. 10 % contribution of drinking water to the TDI

** see explanations above: theoretical value based on 2 L drinking water intake and 10 % contribution of drinking water to a provisional tolerable daily intake.

In summary:

Alkylphenols were frequently detected in surface waters. Nonylphenols occur in the µg/L range , Octylphenol was detected at lower concentrations in the ng/L range. Based on the presented data, a great variation of Nonylphenol concentrations in the European surface waters can be assumed due to different efficiencies in the purification of the sewage treatment plant discharging into the river. For groundwater and drinking water little information is available. The reported concentrations are in the ng/L range. Tap water samples analysed in the case study contained no Nonylphenol and no Octylphenol.

It can be concluded from the existing data, that drinking water contributes to a minor part to exposure of humans to alkylphenols.
Based on the current assessment concerning effects on humans, the Nonylphenol quantity consumed via drinking seems to be unlikely to pose an adverse health threat to humans.

However, in order to determine drinking water standards and to evaluate the relevance of the presented Nonylphenol concentrations for humans, more toxicological data as well as more reliable drinking water concentrations of Nonylphenol are essential.

5.4 Synthetic and natural estrogens

5.4.1 Synthetic estrogen 17α-Ethinylestradiol

5.4.1.1 Occurrence

The synthetic estrogen 17α-Ethinylestradiol is the most frequently used active ingredient in the contraceptive pill, and the therapeutic dose is around 30 µg/day (10 - 50 µg/day) (Arzneiverordnungsreport 1998). Its estrogenic potency is comparable to that of the natural estrogen 17β-Estradiol and it belongs to the most potent estrogens. In contrary to the natural hormone 17β-Estradiol, the synthetic hormone 17α-Ethinylestradiol is only slowly biodegradable in sewage treatment works and the environment (Norpoth et al. 1973, Tabak et al. 1981, Ternes et al. 1999, Jürgens et al. 2002). Therefore, even low concentrations of this synthetic hormone may be of concern, if detected in food and drinking water intended for human consumption.

Up to 5 ng/L of 17α-Ethinylestradiol were reported in **surface waters**. In **groundwater** besides negative result around 0.6 ng/L were reported. No information on the occurrence of 17α-Ethinylestradiol raw water was available in the literature.
None of the 5 waterworks monitoring 17α-Ethinylestradiol found it neither in the raw waters nor in the **drinking water**.
In the case study of this project, the tap water samples of the four waterworks were all negative. The drinking water concentrations retrieved from literature and the questionnaires from the waterworks, indicated that most results were negative, but for few drinking waters concentrations of free 17α-Ethinylestradiol up to 1.4 ng/L were reported in literature (Table 18). To verify these relatively high concentrations found in drinking water, further investigations are recommended.

5.4.1.2 Exposure of humans

Estimating a worst-case exposure for 17α-Ethinylestradiol using the maximal reported concentration of the free hormone of 1.4 ng/L and 2 L drinking water consumption per day, a daily intake of 2.8 ng can be calculated for an adult, corresponding to an exposure level of 0.047 ng/kg body weight per day. The exposure of an infant would be higher by a factor of 4.5, due to the higher ingestion of drinking water relative to the body weight (see above). An infant would be exposed to 1.1 ng 17α-Ethinylestradiol per day or 0.21 ng/kg body weight per day, respectively (Table 34).
Based on a common contraceptive pill with a daily dose of 30,000 ng/day, the indirect worst-case exposure of an adult via drinking water is nearly 10 500 times less than the daily therapeutic dose. Concerning the more susceptible part of the population, the daily exposure of an infant would be around 2400 fold less than the therapeutic dose per kg body weight. At present, the physiological relevance of these findings is uncertain. There is no TDI value from the WHO available. Regarding exposure via food, Christensen (1998) estimated the exposure to 17α-Ethinylestradiol from diffuse intake via food and drinking water. He

compared the ingested level with male endogenous estrogen production and found, that the risk was “negligible”.

In summary:

17α-Ethinylestradiol can occur in surface waters at concentrations of a few ng/L. There are limited data regarding the occurrence in groundwater. In drinking water 17α-Ethinylestradiol was neither detected at waterworks nor in the samples of the case study. Low concentrations around 1.4 ng/L reported in the literature seem to be outliers, thus further confirmation is needed.

The contribution of drinking water to the exposure of humans towards 17α-Ethinylestradiol seems to be very low. However, as 17α-Ethinylestradiol is one of the most potent synthetic estrogenic compounds unintentional exposure of the population, especially of developing embryos, prepubertal boys and girls, should be avoided in any case.
For a European wide assessment of the exposure of humans via drinking water, more reliable data on the concentrations in raw water and drinking water are required.

5.4.2 Natural estrogens 17β-Estradiol and Estrone

5.4.2.1 Occurrence

The natural estrogen 17β-Estradiol is synthesised in all female vertebrates and to a lower extent in males. The natural estrogens, 17β-Estradiol and the main metabolites Estrone and Estriol, reach the environment via the sewer system or animal excretion (manure). These substances are biodegraded in sewage treatment works and the environment, although the speed of degradation is often too slow to allow complete removal before the substances reach water courses. In Table 32 mean values for the excretion of natural estrogens by humans are shown (data taken from W. Jülich 2000). The amount of the excreted natural estrogens differs depending on age and gender. Highest amounts are excreted during pregnancy.
Table 33 summarises the concentrations of 17β-Estradiol in human blood and shows the internal exposure of humans. For the population of The Netherlands (16 millions), for example, Jülich (2000) calculated a mean value of 210 µg estrogens per inhabitant per day resulting in 3.2 kg estrogens per day for the whole country. Additionally, 17β-Estradiol and other natural estrogens are prescribed as therapeutical for menopausal disorders with a daily dose of around 2 mg. Regarding the German population, around 823 kg estrogens were ingested therapeutically in 1996 (based on data of the German Arzneimittelreport 1998 and Rote Liste 1999). Assuming 140 L sewage per inhabitant, 81,3 million inhabitants and 90 % degradation of the estrogens by human metabolism and in sewage treatment plants, an emission of 2 ng/L estrogens in sewage effluents can be calculated. Those concentrations had been detected in environmental samples (Table 11).

17β-Estradiol was detected in **surface waters** in Germany, Italy, The Netherlands and the United Kingdom up to 12 ng/L. Most of the concentrations reported in literature and in the reports from authorities ranged from < LOD to around 4 ng/L. Maximal values were reported between 5.5 and 12 ng/L. Estrone, a main metabolite, was found at concentrations up to 17 ng/L, whereas the maximal value seems to be very high. Except the latter value, Estrone concentrations in surface waters were between < LOD and 8 ng/L. In **groundwater** besides negative results concentrations of maximal 15 ng/L Estrone were reported. Free 17β-Estradiol was not found in groundwater.

In the **drinking water** samples of the case study, no estrogens were detected. In the questionnaires, only one waterworks reported Estrone concentration of 1 ng/L. In recent publications, 17β-Estradiol and Estrone were analysed in drinking waters, up to 2.1 ng/L 17β-Estradiol and 0.6 ng/L Estrone. Because the reported amount of 17β-Estradiol is in the range of concentrations found in surface water, these findings needs further confirmation.

5.4.2.2 Human exposure

Beside the intentional ingestion of natural estrogens as a pharmaceutical, diffuse unintentional exposure can occur via food and drinking water.
Estimating a worst-case exposure using the maximal reported 17β-Estradiol concentration of 2.1 ng/L and 2 L drinking water consumption per day, a daily intake of 4.1 ng can be calculated for an adult, corresponding to an exposure level of 0.068 ng/kg body weight per day. An infant would be exposed to 1.6 ng 17β-Estradiol per day equal to 0.32 ng/kg body weight per day, respectively (Table 34).

Potential exposure via drinking water should be compared to the dietary intake of estrogens. The occurrence of sex steroid hormones in food based on a survey of the German market basket was analysed by Hartmann et al. (1998) and Fritsche and Steinhart (1999) (Table 34). They estimated a daily dietary intake of 0.1 µg estrogens per day. Milk products supplied about 60 - 80 % of ingested female sex steroids.
Compared to this, the roughly estimated ingestion of estrogens with drinking water seems to contribute with a maximum of around 4 % to the total exposure of an adult via mixed diet.

The daily 17β-Estradiol production of males exceeds this daily intake via food by factors of 400 and 1300 and that of infants by factors between 10 and 400. Furthermore, natural steroid hormones show only low oral activity because around 90 % of the ingested hormones are inactivated by the first-pass mechanisms of the liver. Fritsche and Steinhart (1999) concluded that hormonal effects cannot be expected from the ingestion of naturally occurring dietary steroid hormones.
The FAO/WHO Expert Committee on Food Additives (JECFRA) estimated an ADI value for 17β-Estradiol of 0.05 µg/kg body weight per day. Compared to this value the exposure of consumers to 17β-Estradiol is lower by a factor between 250 and 1000.

Based on this knowledge, effects on humans due to the intake of steroid hormones in drinking water is not expected. However, for a reliable evaluation of the exposure of consumers more reliable data are needed.

In summary:

17β-Estradiol can be present in surface waters in the lower ng/L range and in ground-water below 1 ng/L.
In the case study generally no 17β-Estradiol and Estrone was detected. In literature and questionnaires from waterworks Estrone concentrations in drinking water up to 2 ng/L were reported. Due to the reported treatment trail, this high value is non plausible and needs confirmation.

Compared to an average dietary intake of estrogens via food, the roughly estimated ingestion of estrogens with drinking water seems to contribute around 4 % to the total exposure of an adult via mixed diet.
Based on this knowledge, effects on humans due to the intake of steroid hormones in drinking water is not expected. However, for an evaluation of the exposure of consumers more reliable data are required.

Table 32: Excretion of Estrogens by humans (Jülich 2000).

population	excretion [estrogens µg/day]	mean excretion [estrogens µg/day]
infants	1 – 40	20
males	40 –130	85
female, not pregnant	50 – 450	250
female, pregnant	450 – 30 000	10 000
female, post menopausal	5 - 50	28

Table 33: Concentrations of 17β-Estradiol in human blood

population	[ng/L blood]	reference
prepubertal boys	0.08 1.44 +/- 0.87	Klein et al. 1994 Paris et al. 2002
prepubertal girl prepubertal girls	0.6 ± 0.6 3.53 ± 2.33 0.90 ± 0.95	Klein et al. 1994 Paris et al. 2002 Klein et al. 1999
girls with premature thelarche	2.29 ± 1.23	Klein et al. 1999
pubertal girl	26.77 ± 18.32	Paris et al. 2002
female	92.2 ± 9	Bahamondes et al. 2000
male	30	Müller et al. 1998.
female, postmenopausal	5.4 ng/L (17β-Estradiol) 21.6 ng/L (Estrone)	Lonning et al. 1997

Table 34: Exposure of humans with Estrogens in food

	hormone	concen-tration [µg/L]	dietary daily intake [µg/day]	daily exposure level [µg/kg bw/day]	% of mixed diet	reference
			via food	**via food**		
Germany (mixed diet) adult	Progesterone		10	0.17		Hartmann et al. 1998
	17ß-Estradiol and Estrone		0.1	0.0017	100 %	
	Testosterone		0.05	0.0008		
Plant sterols in western diets (adult)	Most abundant ß-Sitosterol Campesterol Stigmasterol		160 - 360	2.7 - 6		Hartmann et al. 1998
Isoflavones in soy-based food	mostly Daidzein and Genistein			up to 1000 (adult) up to 8000 (baby)		Degen et al. 1999
			0.25 L per day			
Whole milk	17ß-Estradiol	0.01 - 0.09	up to 0.023	0.00038 (adult)		Fritsche &
	Estrone	0.01-0.12	up to 0.03			Steinhart, 1999
Worst case exposure estimation based on results of this study			**via drinking water (2 L)**	**via drinking water**		
Drinking water literature data	17ß-Estradiol	0.0021	0.0041 (adult) 0.0016 (infant)	0.00007 (adult) 0.0003 (infant)	4 %	this project
Drinking water literature data	17α-Ethinyl-estradiol	0.0014	0.0028 (adult) 0.0011 (infant)	0.00005 (adult) 0.0002 (infant)	3 %	this project

5.4.3 Phytosteroids

Waterworks and authorities were also asked for information regarding the presence of the phytosterols β-Sitosterol and Stigmasterol and the isoflavones Daidzein and Genistein and the Daidzein metabolite Equol in raw waters and drinking water.

From the 51 waterworks which responded to the questionnaire β-Sitosterol was analysed by 2 waterworks. Due to the ubiquitous occurrence of the weakly estrogenic β-Sitosterol in surface waters up to around 1.5 µg/L (Wenzel et al. 1998, Stumpf et al. 1996) the substance was also present in the raw water ranging from 0.06 to 0.78 µg/L. After water treatment, β-Sitosterol was still measured in drinking water samples between 0.01 and 0.02 µg/L. One reason for this might be, that algal microlayers are formed, e.g. on the surface of the activated charcoal, and the dissolving algae release the compound. The other phytoestrogens were not analysed by the waterworks.

Genistein and Daidzein were detected by Ternes et al. (2001) in some effluents of sewage treatment plants up to 0.017 µg/L. Stigmasterol was detected in a higher number of effluents up to 1.5 µg/L. In surface waters flavonoids were detected only in trace amounts with low frequencies: Genistein was not detected in the 10 investigated samples, Equol was detected up to 0.03 µg/L. Stigmasterol up to 0.08 µg/L and β-Sitosterol up to 0.34 µg/L. It should be noted that only the dissolved compounds were measured, since the samples were filtered prior to analysis to avoid the proportion leaching out of phytoplankton. While isoflavones were not detected in drinking water, Stigmasterol and β-Sitosterol were present up to 0,066 µg/L.

Humans are mainly exposed to phytoestrogens via food (table 34). The most important phytoestrogens for humans are isoflavones. Calculated daily isoflavone intake for adults is up to 1 mg/kg body weight and in the range of 4.5 –8 mg/kg body weight for babies fed with soy based infant formula (Degen 1999). It is assumed, that isoflavones are beneficial for human health, depending on the dose, by lowering the risk of severe chronic diseases and protect against certain types of cancer (Degen et al. 2002).

It is assumed, that isoflavones will normally not be present in drinking water and that effects may not be expected if trace amounts of isoflavones and their phytosteroids may be ingested via drinking water.

5.5 Potential effects of EDCs in drinking water on human health analysed in the case study

For the health risk assessment of chemicals generally two sources of information are available: toxicity studies using laboratory animals and studies in human populations. Since the number of human studies is limited the evaluation of toxic effects often relies on animal studies only. It is of utmost importance that these animal studies fulfil some basic requirements to facilitate the comparison of animal data with the human situation. Most of the studies provided by industrial laboratories are in compliance with OECD protocols laying down the minimum requirements for several types of toxicity studies (OECD 1987).

To evaluate the toxic effects of chemicals in drinking water main emphasis is placed on animal studies with chronic exposure (lifespan) or subchronic exposure, because concentrations of contaminants are relatively low and the consumption of drinking water normally takes place over the entire life. The studies aim to characterise biochemical, functional and structural changes in a large number of target tissues, such as liver, kidneys, lung, brain, reproductive organs, bone marrow and blood (van Leeuwen 2000).
Regarding disturbances of the endocrine system, chronic studies considering reproductive performance are expected to detect disturbances of the sexual endocrine system. Nowadays, conventional test protocols are modified and enhanced to detect effects on the endocrine system (e.g. hormone levels, oestrus cycle, sperm counts, behaviour).

For the health risk assessment of chemicals with the potential to disrupt the endocrine system emphasis should be placed on one- or two-generation studies with enhanced protocols specifically designed to pick up endocrine disrupter effects. Lifespan exposure is of importance for the exposure of different developmental stages with different sensitivity. Exposure to hormonally active agents can lead to *reversible* effects in adults, whereas early developmental exposure can result in *irreversible* effects (National Research Council 1999).

On the basis of current knowledge, for the EDCs analysed in the case study potential health effects through drinking water can be summarised as follows:

Organotin compounds

From the group of organotin compounds TBT is of most concern, since it is a strong aromatase inhibitor. In acute and chronic animal studies effects of TBT on liver, the haematological and the endocrine system are described. Immunotoxicity is currently regarded as the most sensitive parameter obtained in a study with rats, the NOAEL being 0.025 mg/kg bodyweight (WHO 1990).

As stated in the BGVV opinion (2000), an inhibition of the aromatase in vivo cannot be excluded. But it is assumed that the dose causing inhibition of the enzyme is relatively high. As a consequence, other more sensitive parameters will be predominant rather than respective effects on the sexual hormone system. This aspect will be proven further on. Though TBT is the most active substance within the group of organotin compounds, it should be considered that for some other compounds of this group the toxicological knowledge is limited. An additive effect of compounds with similar mechanism is normally regarded as the most appropriate assumption and a comparable mechanistic profile regarding immunotoxic effects in subchronic and chronic feeding studies with rats was observed for TBT, DBT, DOT and TPT (BGVV 2000). These effects are not associated with aromatase inhibition and therefore are unlikely to be associated with endocrine disruption.

The guideline value of the WHO Guideline (1999) for drinking water derived from the TDI value was set to 2 µg/L TBT, based on 20 % of the TDI allocated to drinking water. In the case study TBT was not detected in drinking water. Moreover, worst case assumptions indicated that a direct health hazard through drinking water is not to be expected.

Bisphenol A

Bisphenol A is estrogenic both in vivo and in vitro. The estrogenic activity in vitro is about 3–5 orders of magnitude weaker than that of 17β-Estradiol. Data on chronic effects on humans are not available, therefore the assessment is based on animal data.
In the Opinion of the Scientific Committee on Food on Bisphenol A (2002) presently available animal studies on developmental toxicity, reproduction and endocrine activity are summarised. Clear evidence of in vivo estrogenic activity of Bisphenol A comes from positive responses in the uterotrophic assay, from studies showing induction of vaginal cornification and vaginal cell proliferation, and other estrogenic responses in studies with mice and rats, e.g. increases in the length of dioestrus and oestrus in mature rats. In the uterotrophic assay, different effect levels have been observed depending on the route of administration of Bisphenol A. It is suggested that only the parent compound is estrogenic and that oral exposure is likely to be of lower risk than other routes.

In several studies rats and mice have been exposed in the prenatal and/or early postnatal period, or continuously in one-, two- or three-generation studies. Effects on vaginal opening, prolongation of oestrus, reduction in daily sperm production, and increase in prostate weight and size have been reported, but were often not observed by other laboratories using comparable study designs, or they were considered insufficiently robust for the evaluation of toxic effects on humans. However, the summary of the more recent studies indicates that reproductive and endocrine-related endpoints are important in the risk assessment of Bisphenol A.

The Committee has therefore based the TDI on the results from a recent, comprehensive three-generation study in the rat with enhanced protocols specifically designed to pick up endocrine

disrupter effects. The dose levels ranged between 1 µg/kg bw/day and 500 mg/kg bw/day. There were no effects on fertility and reproduction except a reduction of the total number, and of live pups per litter at birth at the highest dose which also caused adult systemic toxicity. The pivotal effects were significant reductions in adult body weight and pup body and organ weight. The LOAEL was 50 mg/kg bw/day. The overall NOAEL for Bisphenol A is considered to be 5 mg/kg bw/day.
Applying an uncertainty factor of 500 to the NOAEL (10 for interspecies and inter-individual differences each, 5 for uncertainties concerning the data) a temporary TDI of 0.01 mg/kg bw/day was derived by the SCF (2002). The Committee recommends that the temporary TDI be reviewed once significant new data are available.
Based on the current assessment concerning effects on humans, the Bisphenol A quantity consumed via drinking seems to be unlikely to pose an adverse health threat to humans.

Nonylphenol

Nonylphenol is estrogenic both in vivo and in vitro. The in vivo estrogenic activity of Nonylphenol has been assessed in several studies using an assay based upon the uterotrophic response in the rat. The estrogenic activity in vitro and in vivo is about 3-6 orders of magnitude weaker than 17β-Estradiol (EU RAR 2001). As data on chronic effects on humans are not available, the assessment is based on animal data. In the food sector, Nonylphenol is listed in the Synoptic Document in the SCF list 8 (substances for which only scanty and inadequate data were available) (SCF 2002b).

The effects of Nonylphenol on fertility and reproductive performance have been investigated in a multigeneration study based on the OECD guideline for a two-generation reproduction toxicity study (NTP 1997). The rats were exposed via diet to Nonylphenol at doses between 15 and 160 mg/kg bw/ day during a non-reproductive phase and up to 300 mg/kg bw/day during lactation. Among the parameters investigated, oestrus cycle was monitored and at necropsy of adult animals, sperm samples were taken and testicular spermatid counts were made.
There was evidence of general toxicity in adults of all generations at 50 and 160 mg/kg bw/day. Considering the reproduction-related parameters, there were no adverse effects on fertility or mating performance. However, there were changes, although relatively slight, in the oestrus cycle length, timing of vaginal opening, ovarian weight and sperm/spermatid count. As summarised in the Risk Assessment Report (EU RAR 2001), this study provided evidence that Nonylphenol exposure over several generations can cause minor perturbations in the reproductive system of offspring, although functional changes in reproduction were not included at the dose levels tested. The NOAEL for these changes was 15 mg/kg bw/day. Evidence of testicular toxicity was found in another study at dose levels which also cause mortality in a repeated dose gavage study in rats (LOAEL 100 mg/kg bw/day).

It should be noted that the changes found in the reproduction study (NOAEL 15 mg/kg bw/day) occurred at exposure levels in excess of the LOAEL for repeated dose toxicity. In this toxicity study the lowest level for renal toxicity was found to be 15 mg/kg bw/day.

A provisional TDI value of 0.005 mg/kg body weight was derived in the toxicological evaluation of the Danish Institute of Safety and Toxicology by using an uncertainty factor of 3000, (Nielsen et al. 2000). Using this TDI value, a provisional drinking water guidance value of 15 µg/L can be calculated. To improve the preliminary risk assessment for humans more information are required.
However, all reported concentrations of Nonylphenol were significantly lower than the above rough estimation of acceptable drinking water concentrations derived from the currently available toxicity data. Based on the above mentioned information, the Nonylphenol quantity consumed via drinking seems to be unlikely to pose an adverse health threat to humans.

5.6 Identification of EDCs which might be relevant for future regulations in drinking water

For an identification of those individual endocrine disrupters or groups of endocrine disrupters that might be relevant for future regulation in drinking water the currently available data are insufficient and do not allow an ultimate decision.

Based on the information compiled in this study, the following substances may be taken into consideration:

Bisphenol A, Nonylphenol, TBT,
and the synthetic and natural estrogens 17α-Ethinylestradiol, 17β-Estradiol and Estrone.

The suggestion was based on the frequency and probability of their occurrence in raw water and drinking water of waterworks as well as on their endocrine disrupting activity. Though synthetic and natural estrogens occur at very low concentrations, they were suggested to be regulated since they are the most potent estrogenic compounds currently known.

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APPENDIX 1 QUESTIONNAIRES

A 1.1 Questionnaire for National Authorities

SET-Questionnaire for Authorities (**EUContract No B3-4305/2000/308717/MAR/D1)**

Fraunhofer–Institute for Environmental Chemistry and Ecotoxicology Dr. Andrea Wenzel Dr. Monika Herrchen, Dr. Peter Lepper Grafschaft, Auf dem Aberg 1 D-57392 Schmallenberg Telefon +49 2972 302 329 – 215, -224 Telefax +49 2972 302 319 e- mail: wenzel@iuct.fhg.de internet: www.iuct.fhg.de	**ESWE-Institute for Water Research and Water Technology GmbH** Dr. Thomas Ternes, Dipl.-Ing. Jeannette Stüber Söhnleinstraße 158 D-65201 Wiesbaden-Schierstein

Questionnaire for Authorities concerning Endocrine Disrupting Chemicals (EDCs)

The current questionnaire is part of an EU-contract with the ESWE-Institute for Water Research and Water Technology as well as for the Fraunhofer Institute for Environmental Chemistry and Ecotoxicology *(EU-Contract No B3-4305/2000/ 308717/ MAR/D1)*. The main objective of the contract will be to elucidate potential sources which may lead to a contamination of drinking water with endocrine disrupting compounds (EDCs). All your data will be considered ***confidential*** and will be ***anonymously*** evaluated. On request, we will be pleased to provide you with the final project report after its approval by the EU.

For evaluation of the questionnaire outcome we want to establish an information network. We would like to include Deputies from water suppliers, as well as scientists involved in any research work on drinking water quality and techniques, but also representatives of Commission services concerned and public institutions further EU institutes. The idea of the network will be to establish a platform for exchange of information and to involve you in decision processes. If you are interested in those panel work, please make a cross at the bottom of this page, close to your contact address.

We kindly ask you to answer - as many as possible - the following questions. For requirements to fill in the form do not hesitate to contact the following persons (Dr. Andrea Wenzel, Dr. Monika Herrchen). Please, return the form to the Fraunhofer-Institute within 5 weeks **(not later than 31th of December)**, even if you are unable to answer all questions. Thank you very much in advance for your co-operation!

The following information will be used only for requests to you by phone or email:

1. Name and specific address of the authority:

2. Contact person:
   name: phone:
   fax: e-mail:

   I am interested in the work in the information network: YES NO

We will keep all answers in confidence! Side 1 of 4 ; Version 0810100501

SET-Questionnaire for Authorities (**EU Contract No B3-4305/2000/308717/MAR/D1)**

Data requested for raw water and drinking water

Do you have any data about following substances and compounds? If yes, please fill in. For insertion of information relating to the concentrations (sample site, year, analytical method, limit of detection), please modify and/or copy the table.

You can also send us data in other formats (Word and Excel files are preferred, hardcopies are also welcome).

Group	Substance	concentration in Groundwater [µg/L]	concentration in Drinking Water [µg/L]	concentration in other Raw Water # [µg/L]	concentration in Surface Water [µg/L]	retrieved for COMMPS 1998***	analyses r or s +	analytical methods / Limit of detection
Biocides								
	TBT					yes		
	TBTO							
	o-Phenyphenol							
Pesticides								
	Acetochlor							
	Alachlor					yes		
	Amitrol							
	Atrazine					yes		
	Chlorfenvinphos					yes		
	Diuron					yes		
	Endosulfan					yes		
	Heptachlor					yes		
	Isoproturon					yes		
	Lindan					yes		
	Linuron							
	Maneb							
	Metam Natrium							
	Nitrofen							
	Simazine					yes		
	Thiram							
	Vinclozolin					yes		
	Zineb							

Group	Substance	concentration in Groundwater [µg/L]	concentration in Drinking Water [µg/L]	concentration in other Raw Water [µg/L]	concentration in Surface Water [µg/L]	retrieved for COMMPS 1998***	analyses r or s	analytical methods/ Limit of detection
Environmental Chemicals								
	Styrene							
	Nonylphenol (technical mixture)					yes		
	4-Nonylphenol (branched)					yes		
	Nonylphenol ethoxylates					yes		
	4-tert-Octylphenol					yes		
	3,4-Dichloroanilin					yes		
	Bisphenol A					yes		
	Benzo(a)pyrene					yes		
	Phthalates*, which?					yes		
	Benzophenones							
	Phenolic compounds							
	4-Nitrotoluene							
	Resorcinol							
	POPs**, which?					yes		
Further.								

We will keep all answers in confidence! Side 3 of 4 ; Version 081001

SET-Questionnaire for Authorities (**EUContract No B3-4305/2000/308717/MAR/D1)**

Group	Substance	concentration in Groundwater [µg/L]	concentration in Drinking Water [µg/L]	concentration in other Raw Water [µg/L]	concentration in Surface Water [µg/L]	retrieved for COMMPS 1998***	analyses r or s	analytical methods/ Limit of detection
Hormones								
	Daidzein							
	17α-Ethinylestradiol							
	17β-Estradiol							
	Estrone							
	Estriol							
	Equol							
	Genistein							
	Testosterone							
	Tamoxiphen							
	β-Sitosterol							
	Stigmasterol							
further...								

\+ RW: Raw Water; DW: Drinking Water r: routine work; s: spot check
#´ Examples for raw water: spring water / lake water / augmented groundwater /reservoir water / water from river bank filtration

* Phthalate (e.g. Benzylbutylphthalate, Diethylhexylphthalate)
** POPs (Persistent Organic Pollutants): Aldrin, Chlordan, Dieldrin, Dioxine, DDT, Endrin, Furane (PCDFs), Heptachlor, Hexachlorbenzol, PCBs, Toxaphene,
*** Final report: http://www.iuct.fhg.de/commps/

We are very much pleased to receive research and analytical reports dealing with endocrine disrupters.

Thank you very much for your help!

A 1.2 Questionnaire for waterworks

SET-Questionnaire for Waterworks (**EUContract No B3-4305/2000/308717/MAR/D1)**

ESWE-Institute for Water Research and Water Technology GmbH Dipl.-Ing. Jeannette Stüber, Dr. Thomas Ternes Söhnleinstraße 158 D-65201 Wiesbaden-Schierstein Telefon +49 611 780 4390 Telefax +49 611 780 4375 e- mail: Jeannette.Stueber@ESWE.com internet: www.uni-mainz.de/~eswe/ www.eu-poseidon.com	Fraunhofer –Institute for Environmental Chemistry and Ecotoxicology Dr. Andrea Wenzel Grafschaft, Auf dem Aberg 1 D-57392 Schmallenberg

Questionnaire for waterworks concerning EDCs

The current questionnaire is part of an EU-contract with the ESWE-Institute for Water Research and Water Technology as well as for the Fraunhofer Institute for Environmental Chemistry and Ecotoxicology *(EU-Contract No B3-4305/2000/ 308717/ MAR/D1)*. The main objective of the contract will be to elucidate potential sources which may lead to a contamination of drinking water with endocrine disrupting compounds (EDCs). All your data will be considered ***confidential*** and will be ***anonymously*** evaluated. On request, we will be pleased to provide you with the final project report after its approval by the EU.

For evaluation of the questionnaire outcome we want to establish an information network. We would like to include Deputies from water suppliers, as well as scientists involved in any research work on drinking water quality and techniques, but also representatives of Commission services concerned and public institutions further EU institutes. The idea of the network will be to establish a platform for exchange of information and to involve you in decision processes. If you are interested in those panel work, please make a cross at the bottom of this page, close to your contact address.

We kindly ask you to answer - as many as possible - the following questions. For requirements to fill in the form do not hesitate to contact the following persons
(Dipl.-Ing. Jeannette Stüber, Dr. Thomas Ternes). Please, return the form to the ESWE-Institute within 6 weeks **(not later than 19th of November)**, even if you are unable to answer all questions. Thank you very much in advance for your co-operation!

The following information will be used only for requests to you by phone or email:

1. Name and specific address of the waterworks/ the water supply company:
   _____
   _____

2. Contact person:
   Company
   name: _____ phone: _____
   fax: _____ e-mail: _____
   I am interested in the work in the information network: Yes ☐ No ☐

   Responsible person for water analyses
   name: _____ phone: _____
   fax: _____ e-mail: _____

We will keep all answers in confidence! Side 1 of 6; version 0410100501

Characteristics of the Waterworks: (confidential)

EU-country: _____

3. Water delivery to consumers and distributors, overall: _____
   (please make all specifics in million m³/a)
   Year of survey: _____

   Volumes sub-divided into:

   a) own water production without water treatment: _____

   b) own water production with water treatment: _____

   c) purchased drinking water: _____

4. Use of waters: (specified in Mio. m³/ a)
   Year of survey: _____

   a) drinking water: _____

   b) process- or service water (external): _____

   c) water for infiltration (external): _____

   d) other waters, which? _____

5. Raw water: (specified in Mio. m³/ a)
   Year of survey: _____

   a) spring water: _____

   b) real groundwater: _____

   c) augmented groundwater: _____

   d) water from river bank filtration: _____

   e) lake water: _____

   f) river water: _____

   g) reservoir water: _____

   h) other waters, which?: _____ _____

   If you use groundwater for raw-water: Do you know the percentages, which one? (e.g. "real" groundwater, percentage of surface water influence, etc.)

   _____
   _____

6. Do you expect an intake of organic substances/compounds in your feeding water at least at unique situations (for instance into the groundwater flow direction towards your wells or - by a river bank infiltration - upstream of your waterworks)?
   What are the sources?

sewage treatment plant ☐
landfill site ☐
river with elevated quantity of treated sewage ☐
irrigation of treated sewage ☐
pharmaceutical and chemistry industry ☐
settlement area ☐
hospitals ☐
agricultural areas ☐
others, explain?: _____

estimated distance to the water reserve or water catchment area in km:

until 1 km ☐ until 5 km ☐ until 10 km ☐

We will keep all answers in confidence! Side 2 of 6; version 0410100501

7. Do you have information about the percentage of treated sewage in your raw water?
Estimated percentage of treated and raw sewage -rate in %: _____

Questions about geology on-site:

a) kind of the aquifer: joint aquifer ☐ interstitial aquifer ☐
others: _____

b) type of rock at the aquifer: _____
(for example: sand/gravel; clay; quartzite; etc.)

c) What is the age of the water? How old? _____

8. Which kind of materials do you use for water catchment, water treatment, water delivery in your distribution area? Special interests on plastics and synthetic paints (e.g. PVC, Epoxy Resin)

	yes	no	place of action
pipelines, consisting of:			
Polyethylene	☐	☐	_____
PVC	☐	☐	_____
cast iron	☐	☐	_____
steel	☐	☐	_____
asbestos cement	☐	☐	_____
concrete	☐	☐	_____
others, explain?:	_____		

used sealing compounds (e.g. Epoxy Resin, Polyurethane):
_____

lining materials in:	yes	no	place of action
• pipelines, e.g. cement mortar	☐	☐	_____
others: _____			
• water storage tanks, e.g. chlorine latex paints	☐	☐	_____
others: _____			

9. Residues of water treatment processes

How many quantities are incurred per year?: _____ t / a

How do you utilise or dispose these residues?
waste deposit ☐
agricultural ☐
others, how?: _____

We will keep all answers in confidence! Side 3 of 6; version 0410100501

Water Treatment Techniques:
(If you have different water treatment trains, please copy this paper and fill it for each train)

10. Does the plant have separated water treatment trains, e.g. for groundwater and river water? What?

_____

11. Used water treatment processes

		yes	no	
a) sedimentation		☐	☐	
b) flocculation				
with flocculation additives like:				
a. Al- or Fe-sulphate or. Fe-chloride		☐	☐	
Sodium aluminate		☐	☐	
b. PAC (Polyaluminiumchloride)		☐	☐	
c. natural, organic polyelectrolytes (e.g. starch, cellulose, alginates)		☐	☐	
d. synthetic polyelectrolytes e.g.	polyacrylic acid	☐	☐	
	polyacrylamide (PAM)	☐	☐	
e. inorganic polyelectrolytes e.g. activated silica acid		☐	☐	
f. others, which?	_____			
d) slow sand filtration		☐	☐	
e) activated carbon filtration		☐	☐	
f) iron removal/ manganese removal		☐	☐	
g) other filtration, which?:	_____	☐	☐	
h) flotation		☐	☐	
i) softening, which:	_____	☐	☐	
k) ion exchange technology		☐	☐	
l) reverse osmosis		☐	☐	
m) ozonation:	_____	☐	☐	
n) disinfection of drinking water with:				
g. chlorination, e.g. Cl_2, ClO_2, NaOCl, $Ca(OCl)_2$		☐	☐	
h. UV irradiation		☐	☐	
i. others		☐	☐	
o) water hardening:	_____	☐	☐	
p) other water treatment processes		☐	☐	

_____

Water treatment processes in successive order (a to p):

☐ → ☐ → ☐ → ☐ → ☐ → ☐ → ☐

We will keep all answers in confidence! Side 4 of 6; version 0410100501

Routine analysis of the raw water and drinking water

Do you have any data about following substances and compounds? If yes, please fill in.

Group	Substance	concentration in Raw Water in µg/l	concentration in Drinking Water in µg/l	analyses r or s	analytical methods
Biozide	TBT				
	TBTO				
	o-Phenyphenol				
Pesticides	Acetochlor				
	Alachlor				
	Amitrol				
	Atrazine				
	Chlorfenvinphos				
	Diuron				
	Endosulfan				
	Heptachlor				
	Isoproturon				
	Lindan				
	Linuron				
	Maneb				
	Metam Natrium				
	Nitrofen				
	Simazine				
	Thiram				
	Vinclozolin				
	Zineb				
Environ-mental Chemicals	Styrene				
	Nonylphenol (technical mixture)				
	4-Nonylphenol (branched)				
	Nonylphenol ethoxylates				
	4-tert-Octylphenol				
	3,4-Dichloroanilin				
	Bisphenol A				
	Benzo(a)pyrene				
	Phthalates*, which?				
	Benzophenones				
	Phenolic compounds				
	4-Nitrotoluene				
	Resorcinol				
	POPs**, which?				
further...					

RW: Raw Water DW: Drinking Water r: routine work s: spot check

next page:

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Group	Substance	concentration in Raw Water in µg/l	concentration in Drinking Water in µg/l	analyses r or s	analytical methods
Hormones	Daidzein				
	17α-Ethinylestradiol				
	17β-Estradiol				
	Estrone				
	Estriol				
	Equol				
	Genistein				
	Testosterone				
	Tamoxiphen				
	β-Sitosterol				
	Stigmasterol				
further...					

RW:Raw Water DW: Drinking Water r: routine work s: spot check

* Phthalate (e.g. Benzylbutylphthalate, Diethylhexylphthalate)

** POPs (Persistent Organic Pollutants): Aldrin, Chlordan, Dieldrin, Dioxine, DDT, Endrin, Furane (PCDFs), Heptachlor, Hexachlorbenzol, PCBs, Toxaphene,

We are very much pleased to receive research and analytical reports dealing with endocrine disrupters.

Thank you very much for your help!

We will keep all answers in confidence! Side 6 of 6; version 0410100501

A 1.3 Results of questionnaires

Table A 1: Results of questionnaires

WW	Water vol. Mill m^3/a	composition of raw water	treatment line	monitored groups	analytical detection	ECDs found in raw water in µg/L	ECDs found in drinking water in µg/L
01	22	river w.	chl/ac/oz/chl	pest/BaP/phenol/POPs	GC	< LOQ	< LOQ
02	93.4	reservoir w.	chl	pest/BaP/phenol/POPs	GC	Endosulfan: 0.0003-0.0045	< LOQ
					GC	Lindane: 0.0004-0.0018	Lindane: 0.0004-0.0015
					LC	BaP: 0.0001-0.0018	BaP: 0.0001-0.0007
03	114.4	groundw./ river w.	chl/fil/oz/ac/chl	pest/alph/styr/BaP	GC	Lindane: 0.052	< LOQ
					GC	< LOQ	Atrazine: 0.033
					GC	Simazine: 0.026-0.122	Simazine: 0.043
					GC	Styrene: 0.07	< LOQ
					GC	Nonylphenol: 1.26-8	Nonylphenol: 0.018-2.1
					GC	4-tert-Octylphenol: 0.2-1.4	4-tert-Octylphenol: 0.09
04	18.03	spring w.	chl/fil/chl	pest/POPs	--	< LOQ	< LOQ
05	16.8	reservoir w.	chl/fil/chl	pest/POPs	--	< LOQ	< LOQ
06	65/75	river w.	chl/fil/ac/chl	pest/styr/alph/BaP/ phenol/POPs/phth	GC	Lindane: 0.009-0.018	Lindane: 0.005-0.008
					GC	Styrene: 0.01-0.025	Styrene: 0.054-0.193
					GC	Nonylphenol: 0.917-3.360	Nonylphenol: 0.014-0.14
					GC	NPEO: 0.187-1.115	NPEO: 0.008-0.113
					HPLC	BaP: 0.0025-0.05	< LOQ
					GC	BBP: 0.415-0.84	BBP: 0.271-0.289
					GC	DHEPRP: 0.76-1.315	DHEPRP: 0.56-0.815
					GC	DEHP: 1.1-2.86	DEHP: 0.915-1.217
07	178/176	reservoir w.	chl/ac/chl	pest/styr/alph/BaP/ phenol/POPs/phth	L/L-GC/ECD	Lindane: 0.008-0.012	Lindane: 0.005-0.011
					L/L-GC/MS	Styrene: 0.01-0.038	Styrene: 0.016-0.057
					L/L-GC/MS	Nonylphenol: 0.015-0.064	Nonylphenol: 0.003-0.031
					L/L-GC/MS	BBP: 0.013-0.032	BBP: 0.007-0.021
08	9.8	reservoir w.	oz/fil/chl	pest/POPs	--	--	< LOQ
09	9.76	groundw./ desalination	chl	pest/BaP/endrin	GC	--	< LOQ
10	123.9/105.3	reservoir w.	chl/oz/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	< LOQ	< LOQ
11	263/287	reservoir w.	chl/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	--	< LOQ
12	1.88/2.33	reservoir w.	chl/oz/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	< LOQ	< LOQ

Table A 1: Results of questionnaires (continued)

WW	Water vol. Mill m^3/a	composition of raw water	treatment line	monitored groups	analytical detection	ECDs found in raw water in µg/L	ECDs found in drinking water in µg/L
13	14.08/14.84	reservoir w.	chl/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	< LOQ	< LOQ
14	10.49/11.59	reservoir w.	chl/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	< LOQ	< LOQ
15	3.3/4.2	reservoir w.	chl/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	< LOQ	< LOQ
16	0.83/0.94	reservoir w.	chl/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	< LOQ	< LOQ
17	80/68	reservoir w.	chl/oz/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	< LOQ	< LOQ
18	15.5/36.4	reservoir w.	chl/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	--	< LOQ
19	36/40	reservoir w.	chl/fil/chl	pest/BaP/phenol/POPs	HPLC/GC-ECD	--	< LOQ
20	14.9	reservoir w.	fil/chl	pest/BaP/POPs/PCBs	--	--	< LOQ
21	10	reservoir w.	oz/chl/fil/chl	pest/BaP/POPs/PCBs/ Benzophenones	GC/MS/MS	--	Lindane: 0.002
22	11.3	groundw./ augm.gw.	chl/ac/chl	pest/POPs	SPE-GC/MS	--	< LOQ
23	24.5	river w./ reservoir w.	chl/fil/chl	pest/BaP/POPs	--	< LOQ	< LOQ
24	14	reservoir w.	fil/chl/f	pest/BaP/POPs/PCBs	GC	--	< LOQ
25	10.78	river w./ groundw.	po/ac/chl	pest/BaP	--	--	< LOQ
26	31.5	reservoir w.	oz/chl/fil/chl	pest/BaP/POPs	SPE-GC	< LOQ	< LOQ
27	54.4	reservoir w.	po/fil/oz	pest/BaP/POPs/PCBs/pc	HPLC/GC	--	< LOQ
28	54.4	reservoir w./ groundw./ augm. gw./ river w.	po/fil/chl	pest/BaP/pc/POPs/PCBs	HPLC/GC	--	< LOQ
29	9.22	river w./ spring w.	oz/chl/oz	pest/alph/BisA/BaP/ pc/POPs	GC/MS	< LOQ	2.0 total Phthalates
30	57	ground w./ bank fil.	ac	pest/biozide/horm/ alph/BisA/BaP/POPs	GC - MS	Atrazine: <0.05-0.1	< LOQ
31	73.7	reservoir w./ bank fil./ groundw.	chl/fil	pest/BaP/pc/POPs/PCBs	GC - MS	Atrazine: 0.01	< LOQ
32	91.5	groundw./ riverw./ spring w.	fil/ac/oz/chl	pest/horm/alph/ BisA/BaP	GC/MS SPE-GC/MS SPE-GC/MS SPE-GC/MS	Atrazine: 0.1 NPEC: 0.22 Estrone: < LOQ b-Sitosterol: 0.78	Atrazine: 0.02 <LOQ Estrone: 0.001 17β-Sitosterol: 0.02

Table A 1: Results of questionnaires (continued)

WW	Water vol. Mill m^3/a	composition of raw water	treatment line	monitored groups	analytical detection	ECDs found in raw water in µg/L	ECDs found in drinking water in µg/L
33	9.1	groundw.	ac/chl	pest	GC/MSD	< LOQ	< LOQ
34	4.2	bank fil.	ac/chl	pest	GC/MSD	< LOQ	< LOQ
35	5.1	groundw.	chl	pest	GC/MSD	< LOQ	< LOQ
36	80	groundw./ bank fil./ lake w./ augm.gw.	fil/oz/fil/ac/chl	pest/alph/BaP/POPs/ pharm	-- -- -- -- -- --	Atrazine: 0.05 (max) Diurone: 0.35 (max) Isoproturone: 0.14 (max) Lindane: 0.02 (max) Simazine: 0.03 (max) BaP: 0.01 (max)	< LOQ < LOQ < LOQ < LOQ < LOQ < LOQ
37	2	groundw./ bank fil.	only ir	pest/BaP/POPs/BTEX/ PAH/LHKW	DIN EN ISO 11369F12 EPA 8270 DIN EN ISO 10301-F4 DIN 38407-F2	< LOQ	--
38	1.9	groundw./ bank fil.	only ir	pest/BaPPOPs/BTEX/ PAH/LHKW	DIN EN ISO 11369F12 EPA 8270 DIN EN ISO 10301-F4 DIN 38407-F2	< LOQ	--
39	116	groundw.	chl	pest/BaP/PCB	--	--	< LOQ
40	24.9	augm.gw	ac	pest/BisA/pharm	-- --	Atrazine: 0.08 BisA: 0.009	< LOQ < LOQ
41	31.3	reservoir w./ bank fil./ groundw.	fil/ac/chl	pest/BaP/PAH/pharm	GC / HPLC -MS	Atrazine: 0.059 (max)	< LOQ
42	11.5	river w.	oz/fil/oz/ac/chl	pest/styr/BaP/pc/ POPs/pharm	DIN EN ISO 11369 DIN EN ISO 6468 DIN EN ISO 38409-F9 DIN 38407-F8 DIN 38409-h-16-i	< LOQ	< LOQ
43	43.5	groundw./ reservoir w.	fil/chl	pest/horm/styr/alph/ BisA/pc/POPs/PCBs	DIN 38407-12 DIN 38407-2 DIN 38409-13-1 DIN 38409-16-2	Diurone: 0.2	Nonylphenol: 0.036 BisA: 0.12
44	4.2	augm.gw.	fil/ac/fil/chl	pest/BaP/POPs/ PCB/horm	SPE - GC/MS	b-Sitosterol: 0.06	β-Sitosterol: 0.01

Table A 1: Results of questionnaires (continued)

WW	Water vol. Mill m^3/a	composition of raw water	treatment line	monitored groups	analytical detection	ECDs found in raw water in µg/L	ECDs found in drinking water in µg/L
45	36	reservoir w./ river w.	oz/ac/fil/chl	pest/alph/BaP/PCBs/ horm/pc	SPE-LC/MS	Atrazine: 0.33	< LOQ
					SPE-LC/MS	Diurone: 0.3	< LOQ
					SPE-LC/MS	Isoproturone: 2.7	< LOQ
					SPE-LC/MS	Simazine: 0.39	< LOQ
					SPE-LC/MS	Nonylphenol: 0.029	< LOQ
46	152	--	chl/fil/ac/chl	pest/styr	GC/MS	Atrazine: 0.01 - 0.5	Atrazine: 0.02
					LC-UV-DAD	Diurone : 0.01 - 0.5	< LOQ
					LC-UV-DAD	Isoproturon: 0.01 - 0.5	< LOQ
					LC-UV-DAD	Linurone: 0.1	< LOQ
					GC/MS	Simazine: 0.01 - 0.1	< LOQ
47	1.8	groundw.	fil/chl	pest	--	--	--
48	3.06	bank fil./ ground w.	chl	pest/BaP/phenol./POPs	DIN EN ISO 11369F12 EPA 8270 DIN 38407-F2	< LOQ	< LOQ
49	5.01	--	fil/chl	pest/BaP	--	Atrazine: 0.022	--
					--	Lindane: 0.015	--
					--	a - Endosulfan: 0.013	--
					--	Diurone: 0.132	--
					--	Isoproturone: 0.112	--
50	18.41	augm. gw.	fil/chl	pest/BaP/phenol./ alph	--	4 - Chlorphenol: 0.05	--
					--	Tribromphenol: 0.03	--
					--	Dimethylphenol: 0.01 - 0.02	--
					--	Diuron: 0.160	--
					--	Benzophenon: 0.054	0.024
51		surface w.	oz/fil	pest/styr/BaP/PAK/ phth	GC/MS	Naphthalene: 0.06	Pyrene: 0.01
						BBP: 0.13	BBP: 0.06
						Disobutylphthalate: 0.16	Disobutylphthalate: 0.06
						Dibutylphthalate: 0.29	Dibutylphthalate: 0.01

Table A 1: Results of questionnaires (continued)

Legend

augm. gw.	augmented groundwater
bank fil.	bank filtration
groundw.	groundwater
lake w.	lake water
reservoir w.	reservoir water
river w.	river water
surface w.	surface water
ac	activated carbon
chl	chlorination
fil	filtration
ir	iron removal
oz	ozonation
po	permanganate oxidation
< LOQ	< limit of detection
--	not filled in

UV	UV detector
DAD	diode array detector
ECD	electron capture detector
GC	gas chromatography
HPLC	high performance liquid chromatography
LC	liquid chromatography
L/L	liquid-liquid extraction
MS	mass spectrometer
MSD	mass selective detector
MS/MS	tandem mass spectrometer
SPE	solid phase extraction

alph	alkylphenols
BaP	benzo(a)pyrene
BBP	Benzylbutylphthalate
BisA	Bisphenol A
BTEX	Benzene, Toluene, Ethylxylol, Xylol
DEHP	Bis(2-ethylhexyl)phthalate
DHEPRP	Bis(2-HEPR)phthalate
horm	hormones
LHKW	Low Volatile Hydrocarbons
NPEC	4-Nonylphenol acetic acid
NPEO	Nonylphenol ethoxylates
PAH	polycycled aromatic hydrocarbons
PCBs	polychlorinated biphenyls
pest	pesticides
pharm	pharmaceuticals
phenol	phenolic compounds
phth	Phthalate
POPs	Aldrin, Dieldrin, Endrin, DDT, Chlordane, Heptachloro
styr	styrene

APPENDIX 2 CHEMICAL AND BIOLOGICAL ANALYSES, METHODS

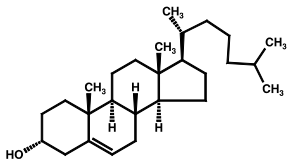
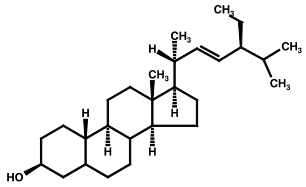
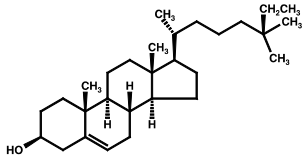
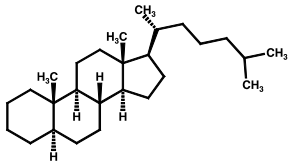
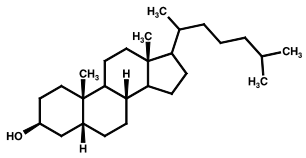
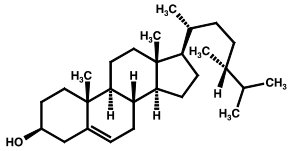
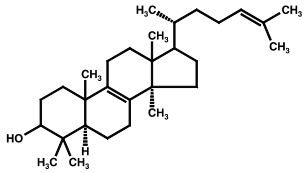
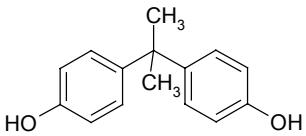
A 2.1 Sampling

The sampling of the waterworks was carried out by the Fraunhofer institute and the ESWE-institute using their own equipment. Only brown ampere bottles were used which had been threefold rinsed successively with acetone and Milli Q-water. They were finally heated at 250 C over night. At each sampling site the sampling was conducted after the water had been running for at least 5 min. Each glass bottle was at least twice rinsed with the water of the respective site. At each site two samples were sequentially taken for the analysis of all analytical groups. In order to minimise the microbial activity, the water for the estrogen analysis and for the YES screen assay were immediately adjusted to pH 2-3 with sulphuric acid (50%, v/v). The water for organotin analyses was adjusted to pH 4 (1 mol/L HCl) and for alkylphenols to pH 2 (4.5 mL 30 % HCl, v/v). During the transport the samples were cooled with an ice bath. The samples were enriched at the next two days, either in Schmallenberg by the Fraunhofer institute or in Wiesbaden by the ESWE institute. For each waterworks blind samples and recoveries were included into the analyses.

A 2.2 Method: Phytosteroids and Bisphenol A

A 2.2.1 Target analytes

Table A 2: Selected phytosteroids and Bisphenol A

Substance	CAS number	Chemical structure	Occurrence
Cholesterol	57-88-5		ingredient of cell membranes, red sea algae, zooplankton, fecal steroid
Stigmasterol	83-48-7		ingredient of plants (e. g. soya beans)
17β-Sitosterol	83-46-5		ingredient of plants (e. g. wheat sprouts)
Cholestan	-		basis for all steroids
Coprostan-3-ol	360-68-9		microbial metabolite of Cholesterol, fecal steroide
Campestrol	474-62-4		ingredient of plant cell membranes
Lanosterol	79-63-0		ingredient of non-photosynthetized plants, fungies, yeasts, intermediates of squales, biosynthesis
Bisphenol A	80-05-7		Plastics industry (production of polycarbonate and epoxy resins)

A 2.2.2 Principle

Phytosteroids and Bisphenol A were determined in aqueous matrices after filtration, solid phase extraction (RP-C_{18}), silica gel clean up and derivatisation by Quadrupol GC/MS.

A 2.2.3 Materials

Internal standard

17β-Estradiol-17-acetate (SIGMA, Deisenhofen Germany) was used as internal reference standard (surrogate standard).

Instrumental parameters

GC/MS instrument	HEWLETT PACKARD 5890 Serie II coupled with HEWLETT PACKARD 5971 Mass Selective Detector
carrier gas	Helium with 85 kPa column head pressure
column	XTI 5; 30 m x 0.25 mm ID; 0.25 µm film, RESTEK Bad Soden
injection	3 µL splitless, 250 °C isotherm
GC temperatures	50 °C isotherm (1.5 min); 20 °C/min to 240 °C; 1.5 °C/min to 290 °C 290 °C isotherm (10 min)

GC/MS parameters

Substance	Retention time [min]	Ion 1 for SIM [m/z]	Ion 2 for SIM [m/z]
Bisphenol A	14.0	358	359
Cholestan	27.1	357	217
Coprostanol	32.9	370	355
Cholesterol	35.9	368	329
Campestrol	39.3	382	343
Stigmasterol	40.4	484	394
Lanosterol	42.1	498	393
β-Sitosterol	42.3	486	396
Bisphenol F (surrogate standard)	13.4	345	179
Mirex (instrumental standard)	21.1	272	237
17β-Estradiol-17-acetate (surrogate standard)	25.3	386	244

A 2.2.4 Analytical method

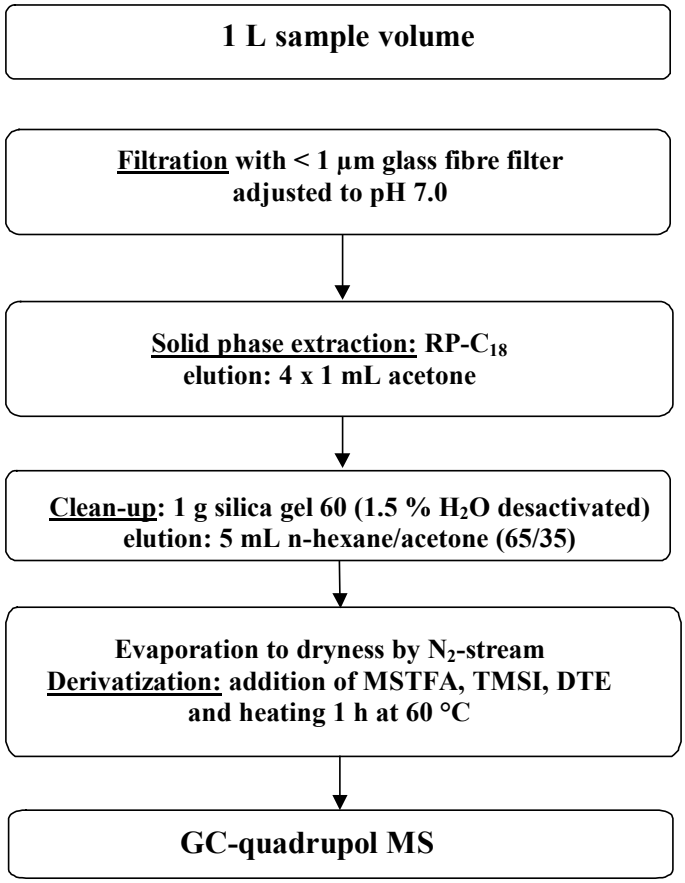


Figure A 1: Analytical scheme: Phytosteroids

A 2.2.4.1 Extraction

The solid phase material (500 mg RP-C_{18} Bulk Sorbent, Separtis GmbH, Grenzahl-Wyhlen, Germany) was filled into glass cartridges which were then conditioned by flushing with 1 x 2 mL hexane, followed by 1 x 2 mL acetone and 3 x 2 mL methanol. The cartridges were washed with 5 x 2 mL of water adjusted to pH 7. 1 L of the water sample adjusted to pH 7 was glass fibre filtered (<1 µm) and spiked with 17β-Estradiol-17-acetate from Sigma (Deisenhofen, Germany) as surrogate standard. Bisphenol F was used as surrogate standard for the analysis of Bisphenol A.
The samples were sucked through the packed glass cartridges at a flow rate of approx. 20 mL/min. Subsequently, the solid phase was dried completely by a nitrogen stream for one hour and the analytes were eluted four times with 1 mL of acetone. The acetone extracts were evaporated to 200 µL by a gentle nitrogen stream.

A 2.2.4.2 Silica gel clean-up and derivatisation

Silica gel clean up and derivatisation were performed as described for the determination of the estrogens (A 2.3.4.2 and A 2.3.4.3).

A 2.2.4.3 Recoveries and limit of quantitation

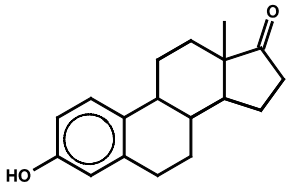
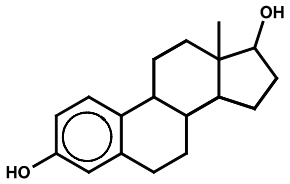
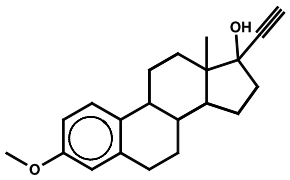
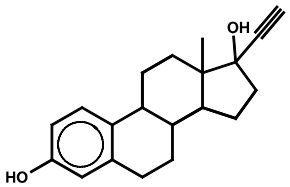
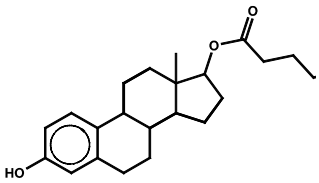
Table A 3: Recoveries and relative standard deviation (RSD) (±1σ, n=3) (spiking with 0,1 µg/L and limit of quantitation (LOQ)

Substance	Recovery [%]	RSD [%]	Rel. Rec. [%]	LOQ [µg/L]
Bisphenol A	102	4	98	0.005
Cholestan	86	12	92	0.01
Coprostanol	83	11	89	0.01
Cholesterol	87	2	94	0.02
Campestrol	83	10	89	0.02
Stigmasterol	95	10	102	0.01
Lanosterol	91	12	95	0.01
β-Sitosterol	88	16	16	0.02

A 2.3 Method: Natural estrogens and contraceptiva

A 2.3.1 Target analytes

Table A 4: Selected natural estrogens and contraceptiva

Substance	CAS number	Chemical structure	Application/ occurrence
Estrone	53-16-7		sexual hormone, natural estrogen
17β-Estradiol	50-28-2		sexual hormone, natural estrogen
Mestranol	72-33-3		contraceptivum, synthetic estrogen
17α-Ethinylestradiol	57-63-6		contraceptivum, synthetic estrogen
17β-Estradiol-17-valerate	979-32-8		synthetic estrogen

A 2.3.2 Principle

Natural estrogens and contraceptive in aqueous matrices were measured in 10 L samples after filtration, solid phase extraction (RP-C_{18}), silica gel clean up and derivatisation by ion trap GC/MS/MS.

A 2.3.3 Materials

Internal standard

17β-Estradiol-17-acetate (SIGMA, Deisenhofen Germany) was used as internal reference standard (surrogate standard).

Instrumental parameters

Gaschromatography

instrument	VARIAN GC 3400
carrier gas	Helium with 60 kPa column head pressure
column	XTI 5; 30 m x 0.25 mm ID; 0.25 µm film, RESTEK Bad Soden with retention gap (1 m x 0.32 mm)
injector	PTV
injection program	50 °C; 100 °C/s to 300 °C; 300 °C isotherm (10 min)
GC temperatures	50 °C isotherm (3.5 min); 20 °C/min to 240 °C; 2 °C/min to 290 °C 290 °C isotherm (10 min)
split flow	approx. 20 mL/min
injection	5 µL splitless
transfer line temperature	280 °C

Mass spectrometry

instrument	VARIAN SATURN 4
ionisation	EI with 70 eV
ion trap temperature	250 °C
collision induced dissociation (CID) RF	120 V
window for MS/MS	3 m/z

MS/MS parameters

Table A 5: Precursor, product ions and retention times used in GC/MS/MS detection

Substance	retention time [min]	precursor ion [m/z]	product ion 1 [m/z]	product ion 2 [m/z]	CID resonant amplitude [V]
Mirex	20.51	272	-	-	-
Estrone (E1)	21.47	342	257	244	0.35
17β-Estradiol (E2)	22.32	416	326	285	0.37
Mestranol	23.37	367	223	193	0.33
17α-Ethinylestradiol (EE2)	24.51	425	231	193	0.35

A 2.3.4 Analytical procedure

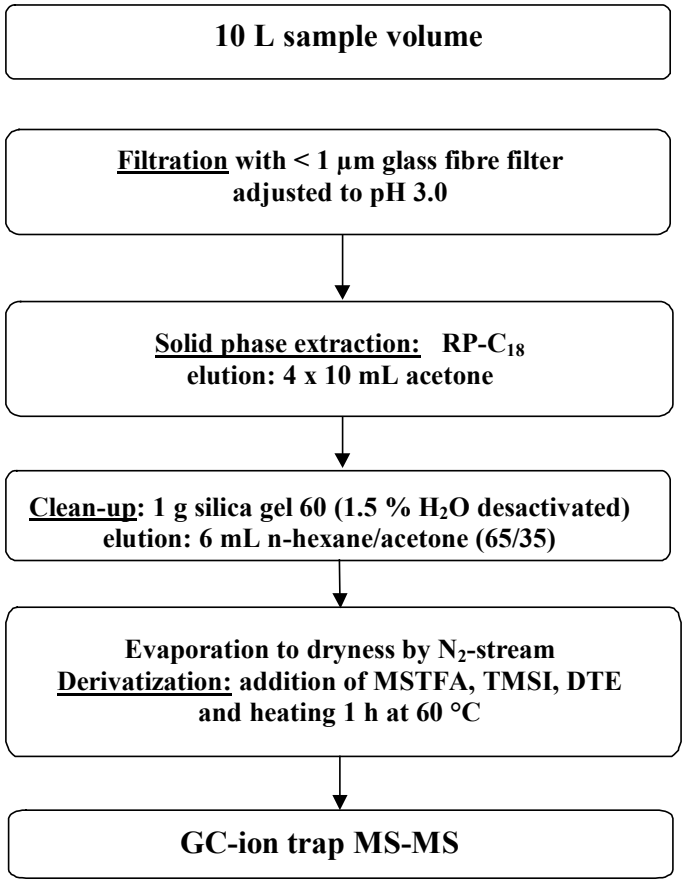


Figure A 2: Analytical scheme: Natural estrogens and contraceptiva

A 2.3.4.1 Extraction

The extraction was performed on a RP-C_{18} disk (Varian) which was conditioned by flushing with 1 x 20 mL hexane, followed by 1 x 20 mL acetone and 3 x 20 mL methanol. The disks were washed with 3 x 20 mL of water adjusted to pH 3. 10 L of the water sample were adjusted to pH 3. The raw water was glass fibre filtered (<1 µm) and all waters were spiked with 17β-Estradiol-17-acetate from Sigma (Deisenhofen, Germany) as surrogate standard. The samples were sucked through the disk at a flow rate of approx. 50 mL/min. Subsequently, the solid phase was dried completely by a nitrogen stream for one hour and the analytes were eluted 4 times with 10 mL of acetone. The acetone extracts were evaporated to 200 µL by a gentle nitrogen stream.

A 2.3.4.2 Silica gel clean-up

1 g of silica gel (silica gel 60, 70-230 mesh, from Merck (Darmstadt, Germany) deactivated with 1.5 % water) was stirred in 4 mL of hexane / acetone (65:35, v/v) and the slurry was manually filled into 6 mL glass cartridges. Before use, the silica gel column was rinsed by flushing with 5 mL hexane+acetone (65+35, v/v). The preconcentrated extracts were quantitatively transferred to the prepared 1 g silica gel column. Finally, the analytes were eluted using 6 mL of hexane+acetone (65+35, v/v) and the sample extracts were evaporated to dryness.

A 2.3.4.3 Derivatisation

For the detection by GC/MS/MS, the extracts were derivatised by adding 50 µl of the derivatisation mixture (N-Methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA)/trimethylsilylimidazole (TMSI)/dithioerytrol (DTE), (1000+2+2; v/v/w). MSTFA and TMSI were purchased from Sigma (Deisenhofen, Germany) and DTE from Merck (Darmstadt, Germany). After a reaction time of 1 h at 60 °C the solution was evaporated to dryness by a gentle nitrogen stream, and the residue was dissolved in 200 µL of hexane. Finally, 100 µg of mirex (from Promochem Wesel, Germany) was added to the final extract as internal standard.

A 2.3.5 Quality assurance

A 2.3.5.1 Determination of recoveries

A volume of 10 L of a groundwater which is not appreciably influenced by man-made organic compounds, were spiked with 0.5, 2 and 10 ng/L of the individual estrogens. The recoveries were determined in relation to a non-enriched standard solution, which was only derivatised. For evaluation, mirex a non-enriched internal standard, was used. SPE extraction, clean-up, derivatisation and detection by GC/MS/MS were performed as described above.

A 2.3.5.2 Calibration and validation

The 10 point calibration was performed during the whole procedure after spiking deep groundwater with the respective estrogens in concentrations ranging from 0.05 to 10 ng/L. In each analysis series, a blank sample of deep groundwater was run in parallel. According to the method evaluation the recovery of the analytes were ≥ 93 % and the relative standard deviation of the method ≤ 14 %. A further quality parameter to ensure that matrix effects did not influence the extraction or clean-up was the ratio between the surrogate standard, 17β-Estradiol-17-acetate and the instrumental standard mirex.

Table A 6: Recoveries of estrogens with different concentrations (0.5 ng/l, 2 ng/l, 10 ng/L), LOQ and LOD

Substances	**Rec. abs. 1 10 ng/L [%]**	**Rec. abs. 2 10 ng/L [%]**	**Rec. abs. 2 ng/L [%]**	**Rec. abs. 0.5 ng/L [%]**	**Rec. rel. (n=2) [%]**	**LOQ [ng/L]**	**LOD [ng/L]**
Estrone	87	88	62	--	103	0.50	0.25
17β-Estradiol	62	66	68	68	75	0.10	0.05
17α-Ethinylestradiol	76	72	74	74	86	0.10	0.05
Mestranol	84	88	82	83	100	0.10	0.05
17β-Estradiol-17-valerate	82	86	75	63	98	0.20	0.10

A 2.4 Method: Iodinated X-ray contrast media

A 2.4.1 Target analytes

Table A 7: Selected iodinated X-ray contrast media

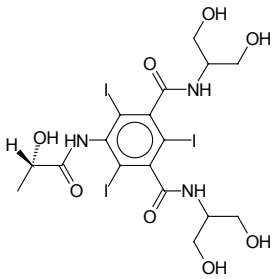
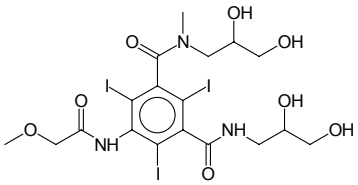
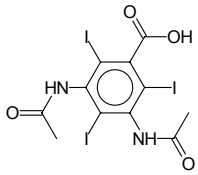
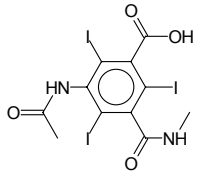
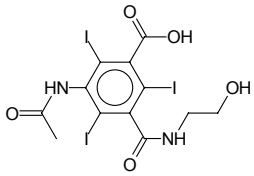
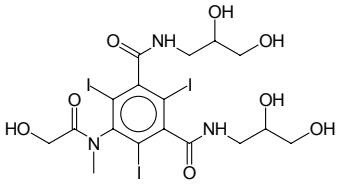
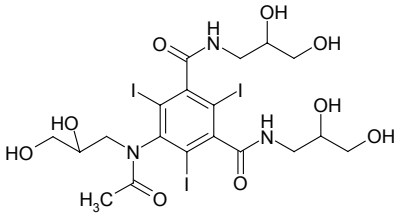
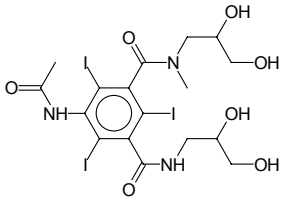
Substance	CAS-number	Chemical structure	Application
Iopamidol	60166-93-0		X-ray diagnostic (non-ionic)
Iopromide	73334-07-3		X-ray diagnostic (non-ionic)
Diatrizoate	131-49-7		X-ray diagnostic (ionic)
Iothalamic acid	2276-90-6		X-ray diagnostic (ionic)
Ioxithalamic acid	28179-44-4		X-ray diagnostic (ionic)
Iomeprol	- -		X-ray diagnostic (non ionic)

Table A 7 (continued)

Substance	CAS-number	Chemical structure	Application
Iohexol	66108-95-0		X-ray diagnostic (non ionic)
Desmethoxy iopromide (DMI); 5-Acetylamino-NN'-bis(2,3-di-hydroxypropyl)-2,4,6-triiodo-N-methylisophthal-amide	76350-28-2		surrogate standard

A 2.4.2 Principle

The iodinated X-ray contrast media were measured in aqueous matrices after solid phase extraction (Isolute® ENV+ material) by liquid chromatography-electrospray tandem mass spectrometer (LC-electrospray tandem MS).

A 2.4.3 Materials

Internal standards

Desmethoxy iopromide (DMI) was used as internal reference standard (surrogate standard).

Instrumental parameters

HPLC

column	125x3 mm, LiChrospher® RP-18(ec), 5 µm, MERCK
alternative column	150x2.1 mm Inertsil® phenyl-3, 3 µm, GL SCIENCES INC.
flow	200 µL/min
column temperature	room temperature
solvent:	water+acetonitrile (92+8, v/v) containing 10 mmol/L ammonium acetate

Mass spectrometry

instrument	LC/MS/MS API 365, PERKIN-ELMER
ionisation	electrospray positive (EI+)
acquisition mode	multiple reaction monitoring (MRM)

MS/MS parameters

Table A 8: Precursor and product ions used in LC/MS/MS-detection using the API 365

Substance	**precursor ions $(M+H)^+$ [m/z]**	**product ions [m/z]**
Iopamidol	777.8	387.1
Iopromide	791.8	572.8
Ioxithalamic acid	644.4	302.2
Diatrizoate	614.6	233.1
Iothalamic acid	614.6	486.7
Iohexol	821.8	804.0
Iomeprol	777.8	405.0
DMI	761.8	543.1

A 2.4.4 Analytical Procedure

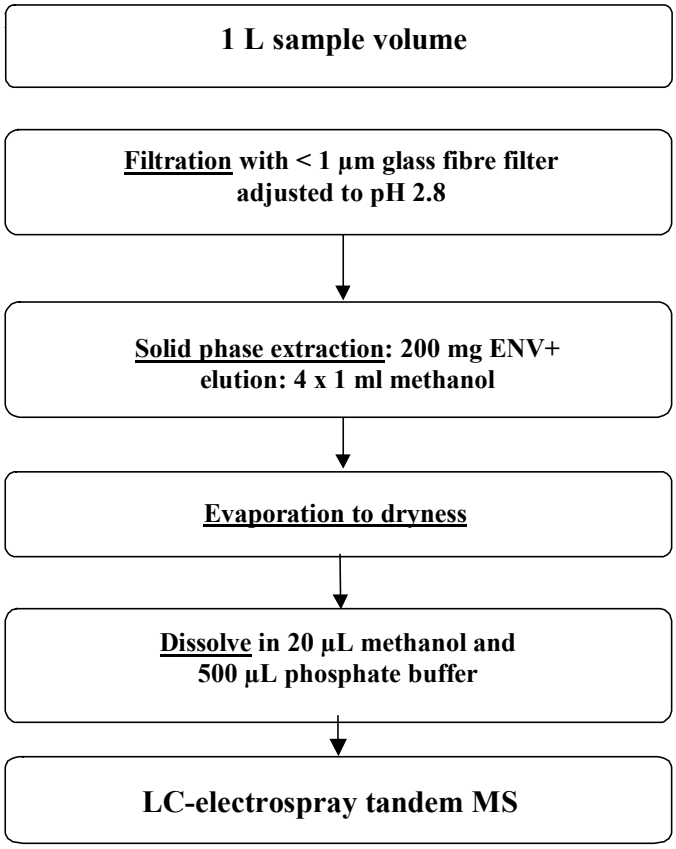


Figure A 3: Analytical scheme: X-ray contrast media

The water sample (1 L) was glass fiber filtered (<1 µm), spiked with the surrogate standard DMI and the pH was adjusted with H_2SO_4 (c=3 mol/L) to 2.8. Then, the sample was sucked with approximately 10 mL/min through a glass cartridge filled with Isolute® ENV+ material. The cartridges were dried for 1 h with nitrogen and eluted 4 times with 1 mL methanol. The extracts were reduced to dryness, then 20 µL methanol and 500 µL phosphate buffer (10 mmol/L Na_2HPO_4 and 10 mmol/L K_2HPO_4) were added. 50 µL of the extract were injected onto the HPLC column.

A 2.4.5 Quality assurance

The limit of quantitation (LOQ) was calculated according to DIN 32645 with a confidence interval of 99 % using the standard deviation of a linear regression curve for a detection range from 0.005 µg/L to 10 µg/L with at least 7 concentrations by spiking groundwater. The LOQ defines the lowest concentration level which allows quantitative analytical determinations. Since the calculated LOQs were always between the first and the second calibration point, the LOQ used were set as the second lowest calibration point of the linear correlation to ensure precise quantitation. The calibration was performed over the whole procedure after spiking groundwater with the standard mixture of X-ray contrast media to attain seven different calibration concentrations.

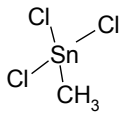
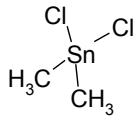
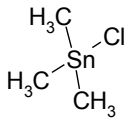
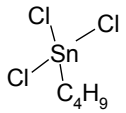
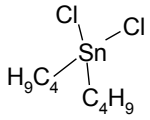
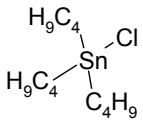
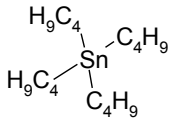
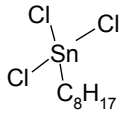
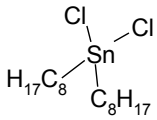
Table A 9: Recoveries of contrast media and LOQ

Substances	**Rec. abs. 1 [%]**	**Rec. abs. 2 [%]**	**Rec. rel. [%]**	**LOQ [µg/L]**
Iopamidol	46	49	54	0.020
Iopromide	95	87	105	0.010
Diatrizoate	86	80	95	0.010
Iothalamic acid	98	101	108	0.020
Ioxithalamic acid	92	90	102	0.020
Iomeprol	74	69	82	0.010
Iohexol	60	53	67	0.020

A 2.5 Method: Organotin compounds

A 2.5.1 Target analytes

Table A 10: Selected organotin compounds

Substance (organotin cation)		**CAS number***	**Chemical structure***	**Application/ occurrence**
Monomethyltin	MMT	993-16-8		Stabiliser in plastics materials
Dimethyltin	DMT	753-73-1		Stabiliser in plastics materials
Trimethyltin	TMT	1066-45-1		Stabiliser in plastics materials
Monobutyltin	MBT	1118-46-3		Stabiliser in plastics materials, surface coatings, catalysts
Dibutyltin	DBT	683-18-1		Stabiliser in plastics materials, surface coatings, catalysts
Tributyltin	TBT	1461-22-9		Biocide
Tetrabutyltin	TTBT	1461-25-2		Chemical industry
Monooctyltin	MOT	3091-25-6		Stabiliser in plastics materials
Dioctyltin	DOT	3542-36-7		Stabiliser in plastics materials

* CAS number and chemical structure for chlorides

A 2.5.2 Principle

Basis of the analytical method was the German guideline DIN 38407-13 (draft 1999-10, 'Determination of selected organotin compounds in water, waste water and sludge by gas chromatography').
Due to the low concentrations expected in the present study the scope of the DIN method of 10 to 1000 ng organotin cation / L was extended to concentrations of about 1 ng/L.
1 L of the water sample was extracted with n-hexane after the addition of hydrochloric acid, internal standard reference substances, sodium diethyldithiocarbamate as complexing and sodium tetraethylborate as derivatisation agent. The alkylated organotin species were cleaned-up by adsorption chromatography (sodiumsulfate/silica gel) and analysed by gaschromatography with atomic emission detection (GC/AED).

A 2.5.3 Materials

Internal standards

The organotin content was calculated according to DIN 38407-13. This means that four internal reference standards were used according to Table A 11.

Table A 11: Internal reference standards used

internal reference standard		**used for calculation of**	
tetrapropyltin	TTPT	tetrabutyltinn	TTBT
tripropyltin	TPT	tributyltin	TBT
		trimethyltin	TMT
diheptyltin	DHT	dibutyltin	DBT
		dioctyltin	DOT
		dimethyltin	DMT
monoheptyltin	MHT	monobutyltin	MBT
		monooctyltin	MOT
		monomethyltin	MMT

Instrumental parameters

Gaschromatography

instrument	GC 5890 Serie II, AGILENT
autosampler	HP 7673 (HEWLETT PACKARD/AGILENT)
capillary column	HP-5, 0.25 µm, 30 m x 0.32 mm
injection temperature	280°C
injection volume	1 µL
splitless	60 s
carrier gas	Helium, constant flow

oven temperatures	40 °C isotherm (3 min); 10 °/min to 220°C; 220 °C isotherm (5 min); 20 °/min to 300°C; 300 °C isotherm (2 min)

Detection

instrument	G2350A, Atomic Emission Detektor, AGILENT
transfer line	HP-5 capillary, 280 °C
cavity temperature	280 °C
measuring wavelength	326 nm

A 2.5.4 Analytical procedure

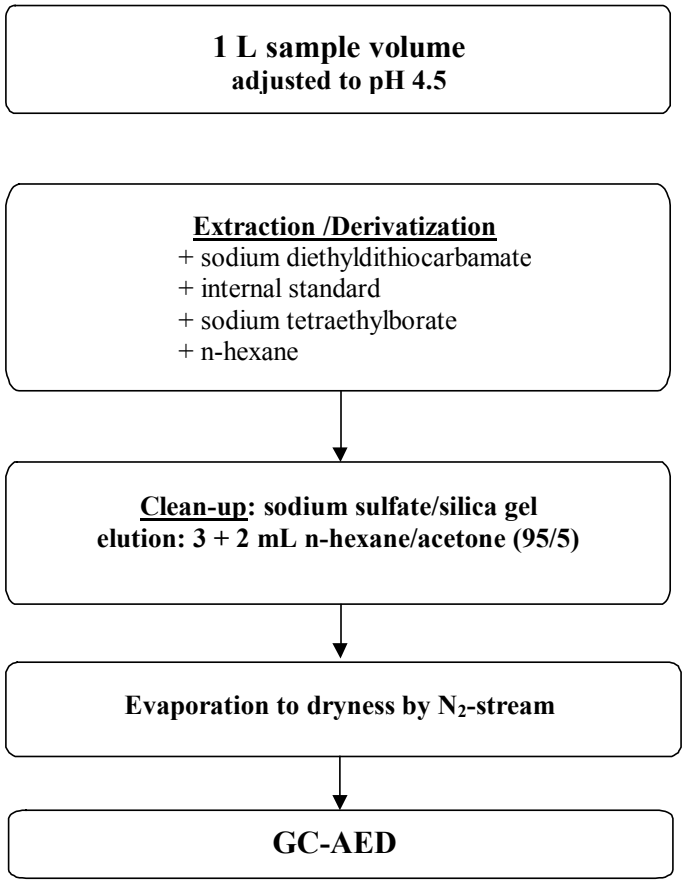


Figure A 4: Analytical scheme: Organotin compounds

A 2.5.4.1 Extraction and derivatisation

The water to be analysed was sampled on site into a precleaned 4 L brown glass bottles and stabilised immediately with 1 M HCl (1.5 mL/L water).

In the laboratory 1 L of the water sample was transferred into a 1 L brown glass bottle and a magnetic stirrer bar was put in (length 6 cm, about 1 cm diameter, triangled). Then 10 mL sodium acetate buffer solution was added to obtain a pH of 4.5. The pH value was controlled and adjusted if necessary using solutions of sodium hydroxide or acetic acid. Then 250 µL of a 0.1 mmolar solution of diethyldithiocarbamate (1.8 g in 20 mL of double destillated water), 250 µL internal reference standard solution (ca. 8 ng/L water), 500 µL of sodium tetraethylborate solution (0.2 g/mL in ethanol) and 20 mL n-hexane were added. After stirring of the samples for 1 h (magnetic stirrer) and a subsequent settling time of 30 min the hexane layer was separated using a Schulze separator and dried in a 100 mL round bottom flask by means 5 g of sodium sulfate.

Then the extract was transferred into a concentration flask and the remaining sodium sulfate reextracted by 5 mL of n-hexane. The extracts were combined and concentrated to about 0.75 mL using a TurboVap II (Zymark) nitrogen evaporation device.

A 2.5.4.2 Sample clean-up

3 g of sodium sulfate was added to a silica gel SPE column (Bakerbond, 1g/6 mL) and rinsed by 5 mL of n-hexane. Then the extract was transferred to the SPE column.

The organotin compounds were eluted from the column by rinsing with 1 x 3 mL and 1 x 2 mL elution solution (5 % acetone in n-hexane, v/v) into a 50 mL flask for use in the nitrogen evaporation device TurboVap II (Zymark) and concentrated to about 0.25 mL (30 °C). The concentrated sample was transferred into a 0.3 mL autosampler vial and the solvent blown off to about 100 µL using a stream of nitrogen. These samples were submitted to gas chromatography and 1 µL each was injected. The organotin compounds were selectively detected using an atomic emission detector.

A 2.5.5 Quality assurance

A 2.5.5.1 Calibration and method limit parameters

The calibration was performed over the total analytical procedure. The results were given as the content of the organotin cation (OTC).

The limits of detection and quantitation were calculated according to DIN 32645 using the SQS98 software (EXCEL Add On Software for the Statistical Control of Analytical Data and Method Validation, Lernhardt und Kleiner 1999). The limit of quantitation (LOQ) was calculated using the calibration line method (DIN 32645) to be about 1.0 ng OTC/L water. The limit of detection (LOD) was determined to be about 0.4 ng OTC / L water.

The relative measuring uncertainty was about 30 % at the LOQ and about 10 % at a concentration of 10 ng OTC / L.

Table A 12: Calibration and method limit parameters

OTC	r^2	RSD %	V_{x0}	LOD ng OTC/L	LOQ ng OTC/L
MMT	0.996	4.3	6.6	0.3	0.7
DMT	0.994	6.0	6.4	0.4	0.9
TMT	0.998	4.0	4.3	0.4	0.9
MBT	0.991	6.5	8.8	0.4	1.0
DBT	0.993	6.2	7.2	0.4	1.0
TBT	0.989	7.9	9.9	0.7	1.6 *
TTBT	0.997	5.5	4.9	0.4	1.1
MOT	0.974	9.4	15.1	0.9	1.9
DOT	0.992	7.2	8.3	0.4	1.0

r correlation coefficient of linear regression
RSD relative standard deviation
V_{x0} relative method standard deviation
LOD Limit of detection
LOQ Limit of quantitation

* The limit parameters of TBT were unusually high in this measuring series. Normally, the TBT values are equal or lower than that of the other organotin compounds.

A 2.5.5.2 Repeatability, blanks and recovery

The analytical instrument was controlled before the measurement of every sample series by analysing a calibration sample prepared at the beginning of the measuring campaign and by a calibration sample prepared by sample processing over the total analytical procedure. In every sample serie a blank processed over the total analytical procedure was analyzed.

In order to examine possible influences of matrix constituents on the results raw and tap water samples of every waterworks were spiked by the addition of a calibration solution. The added amount of the OTC was adjusted to result in an increase of the concentration of the OTCs between 3 to 7 ng OTC/L water.

The recovery rate (Table A 13) was calculated as:

$$\%\ \text{recovery} = \frac{\text{OTC amount after standard addition}}{\text{OTC amount before standard addition} + \text{standard addition}}$$

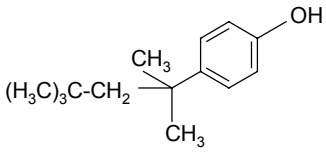
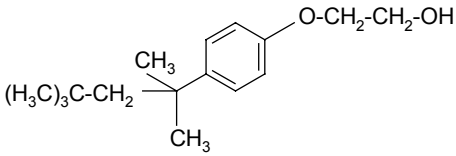
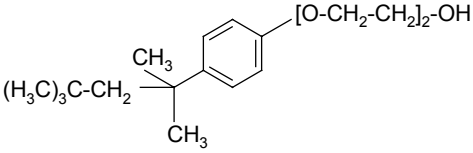
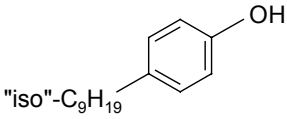
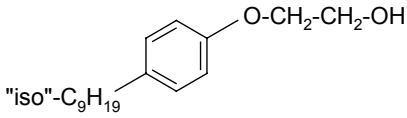
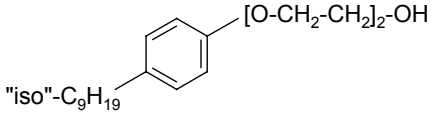
Table A 13: Mean recovery rates in %

	TMT	DMT	MMT	MBT	DBT	TBT	TTBT	MOT	DOT
Waterworks raw and tap water	89	137	121	125	154	115	105	100	158
Laboratory tap water	90	132	123	116	154	106	104	109	145
Laboratory dest. water	103	135	143	125	141	107	73	82	85

A 2.6 Method: Alkylphenols and alkylphenol ethoxylates

A 2.6.1 Target analytes

Table A 14: Selected Alkylphenols (AP) and alkylphenol ethoxylates (APEO)

Substance	CAS number	Chemical structure	Application/ occurrence *)
4-*tert*.-Octylphenol 4tOP	140-66-9		decomposition product of 4-*tert*.-Octylphenol-(poly)-ethoxylates
4-*tert*.-Octylphenol monoethoxylate 4tOP1EO	-		decomposition product of 4-*tert*.-Octylphenol (poly)-ethoxylates
4-*tert*.-Octylphenol diethoxylate 4tOP2EO	-		decomposition product of 4-*tert*.-Octylphenol (poly)-ethoxylates
4-Nonylphenol 4NP (technical isomer mixture)	84852-15-3 (branched chain isomers)		decomposition products of 4-Nonyl phenol(poly)-ethoxylates
4-Nonylphenol-monoethoxylate 4NP1EO (technical isomer mixture)	-		decomposition products of 4-Nonyl phenol(poly)-ethoxylates
4-Nonylphenol diethoxylate 4NP2EO (technical isomer mixture)	-		decomposition products of 4-Nonyl-phenol(poly)-ethoxylates

*) 4-Nonyl-phenol (poly)-ethoxylates and 4-*tert*.-Octylphenol (poly)-ethoxylates are used among other things in the production of tensides, emulsifying agents, synthetic resins or flexibilisers.

A 2.6.2 Principle

A solid phase extraction (SPE) method was used for the enrichment of the APEO from water samples. Initially the samples were acidified with hydrochloric acid to a pH of approx. 2. After addition of the internal standard (IS) solution, the analytes were extracted with SPE on RP-C_{18} cartridges. After cleaning the extracts by adsorption chromatography on silicagel the substances were silylated and measured by GC/MS-MS. The recovery rates over the total analytical procedure were determined by matrix calibration enforced with a raw water sample.

A 2.6.3 Materials

Internal standards

4-n-Nonylphenol (4nNP), 4-n-Nonylphenol monoethoxylate (4nNP1EO) and 4-n-Nonylphenol diethoxylate (4nNP2EO) were used as internal reference standards.

Calibration solutions (multicomponent standards)

Calibration solutions were prepared in two different concentration ranges with 10 concentration levels each.

Range A:	0.25 to 50 ng/L	for 4tOP, 4tOP1EO and 4tOP2EO
	0.5 to 100 ng/L	for 4NP, 4NP1EO and 4NP2EO
Range B:	0.25 to 2.5 ng/L	for 4tOP, 4tOP1EO and 4tOP2EO
	0.5 to 5.0 ng/L	for 4NP, 4NP1EO and 4NP2EO

The concentrations of the internal standards were 10 ng 4nNP/L, 20 ng 4nNP1EO/Land 20 ng 4nNP2EO/L.

Aqueous raw water samples for matrix calibration

Matrix calibration was carried out with a raw water sample, which was provided for subsequent drinking water purification.

After acidifying six 1000 mL portions of the water sample with approx. 1 mL of hydrochloric acid, the analytes and the IS were added with a Pasteur pipette, dissolved in 100 µL of acetone. For this purpose 50 µL of 5 of the calibration solutions (range A) were transferred into 2 mL auto sampler vials. The organic solvent cyclohexane was then evaporated by a gentle stream of nitrogen and the residue was dissolved in 100 µL acetone.

Instrumental parameters

Gaschromatography

instrument	VARIAN 3400
carrier gas	Helium with 100 kPa column head pressure
column	SGE HT8; 25 m x 0,22 mm ID; 0,25 µm film
oven temperatures	155 °C isotherm (1 min); 6 °/min to 320°C; 320 °C isotherm (10 min)
transfer line temperature	290 °C
injector	GERSTEL CIS III
temperature program	60 °C isotherm (0.05 min); 12 °C/s to 280 °C;

	280 °C isotherm (10 min)
split flow	approx. 20 mL/min
auto sampler	GERSTEL MPS2
injection	2 µL MSTFA, split less time: 1.5 min

Mass spectrometry

instrument	FINNIGAN MAT MAGNUM™ / Varian SATURN 4D
ionisation	EI with 70 eV
filament current	70 µA
manifold temperature	200°C

MS/MS parameters

substance	**precursor ions [m/z]**	**product ions [m/z]**	**retention time [min]**
4tOP	207	151/163/179	5.46
4tOP1EO	251	207	10.23
4tOP2EO *)	295	207	14.61
4NP	207/221/235	163/179/193	range 6.35 – 7.72
4NP1EO	251/265/279	207/221/237	range 11.26 – 12.78
4NP2EO	295/309/323	207/221/235	range 15.50 – 16,95
4nNP	179	73	9.55
4nNP1EO *)	179	73	14.49
4nNP2EO	246	133	18.54

*) 4tOP2EO and 4nNP1EO elute in the same acquisition segment

**) technical mixture of approx. 10 isomers which will be separated by capillary gas chromatography

A 2.6.4 Analytical Procedure

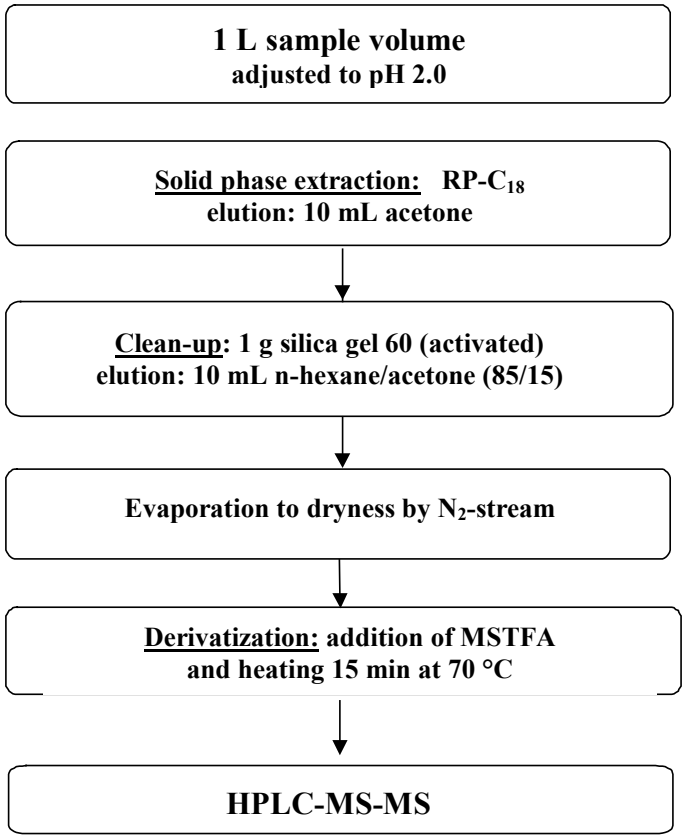


Figure A 5: Analytical scheme: Alkylphenols and alkylphenol ethoxylates

A 2.6.4.1 Sample preparation by Solid-Phase extraction (SPE)

1 L of the water sample was filled in a precleaned 1 L glassbottle and acidified to pH 2 with approx. 1 mL of hydrochloric acid. After adding the IS solution the sample was shaken thoroughly for 1 minute. The enrichment of the analytes was then performed without filtration by manual SPE with the SPE workstation in the following order:

- Conditioning the SPE cartridges with 10 mL of acetone, 10 mL methanol and 15 mL acidified pure water, leave wet
- Loading the sample within approx. 1 hour
- Washing with 10 mL of pure water
- Drying with N_2 for 30 min (pressure 3 bar)
- Eluting the analytes with 10 mL acetone in a 15 mL concentration flask.

The acetone was then evaporated by a gentle stream of nitrogen. The residue was dissolved either in 1 mL n-hexane (method A) or in 1 mL of a mixture of n-hexane/acetone (85/15, v/v) (method B) by ultrasonic treatment.

A 2.6.4.2 Sample clean up

The eluates were partly charged with high amounts of matrix compounds. Therefore, all eluates were cleaned by adsorption chromatography on activated silicagel in a mini chromatography column in the following manner:

- Filling the mini chromatography column with 1 g of activated silicagel
- Washing with 10 mL of hexane/acetone (85/15, v/v)
- Conditioning the silicagel with 10 mL of hexane
- Loading the 1 mL sample, see chapter 'Sample preparation by Solid-Phase extraction (SPE)'
- Washing with 10 mL of n-hexane (only when the residue was dissolved in 1 mL hexane, see chapter 'Sample preparation by Solid-Phase extraction (SPE)', method A)
- Eluting the analytes with 10 mL of a mixture of hexane/acetone (85/15, v/v) in a 15 mL concentration flask.

The organic solvent was then concentrated to approx. 200 µL by a stream of nitrogen. The samples of the waterworks I and II were cleaned using method A (see last chapter), the samples from the waterworks III and IV were cleaned without carrying out the washing step (method B). The different performance of the sample clean-up results in different blank values and consequently in different LOQs (see Table A 15).

A 2.6.4.3 Derivatisation and measurement

To improve the gas chromatographic properties of the analytes, they were converted to their trimethylsilyl ethers by silylation of the OH group with MSTFA. In this derivatisation procedure the MSTFA serves as silylating agent and as organic solvent.

For this purpose the purified eluates were filled in 300 µL GC vials with Pasteur pipettes, and the remaining organic solvent was evaporated to dryness by a stream of nitrogen. After addition of 50 µL MSTFA the vials were closed with a Teflon coated crimp cap. The derivatisation was then completed by heating-up to 70 °C for 15 minutes. After cooling down to room temperature 2 µL were injected into the GC/MS-MS system.

For the basic calibration of the GC/MS-MS system, 50 µL of each calibration solution was transferred into a in 300 µL GC vial and treated as described above.

A 2.6.5 Quality assurance

A 2.6.5.1 Method characteristics

On the basis of the calibration results the method characteristics of the analytical method were calculated according to DIN 32645 using a statistical quality assurance program (SQS 98; Perkin-Elmer). These data are shown in Table A 15.

Table A 15: Method characteristics of the analytical method

compounds	rel. method deviation, V_{xo} [%]	LOQ DIN 32645 [ng/L]	LOQ blank values [ng/L]	recovery rate [%]
4tOP	1.7	**0.5**		99
4tOP1EO	6.8	**0.5**		125
4tOP2EO	7.1	**0.5**		106
4NP (WW I / II)	3.0	(2.5)	**6.0**	112
4NP (WW III / IV)	3.0	(2.5)	**4.0**	112
4NP1EO	4.3	**2.5**		117
4NP2EO (WW I / II)	4.5	(1.5)	**3.0**	100
4NP2EO (WW III / IV)	4.5	**1.5**		100

A 2.6.5.2 Matrix calibration

The method was checked for systematical errors with a standard addition experiment. For this test the basic calibration experiment (only pure standards were analyzed) was compared to a calibration procedure, which was obtained by executing the whole method with matrix charged real water samples. Figure A 6 (a) shows the basic and the matrix calibration functions of 4tOP. Plotting the analyzed amounts of the matrix samples (corrected with the natural present 4tOP content) against the added amounts, the recovery function of 4tOP will be received (see Figure A 6 (b)).

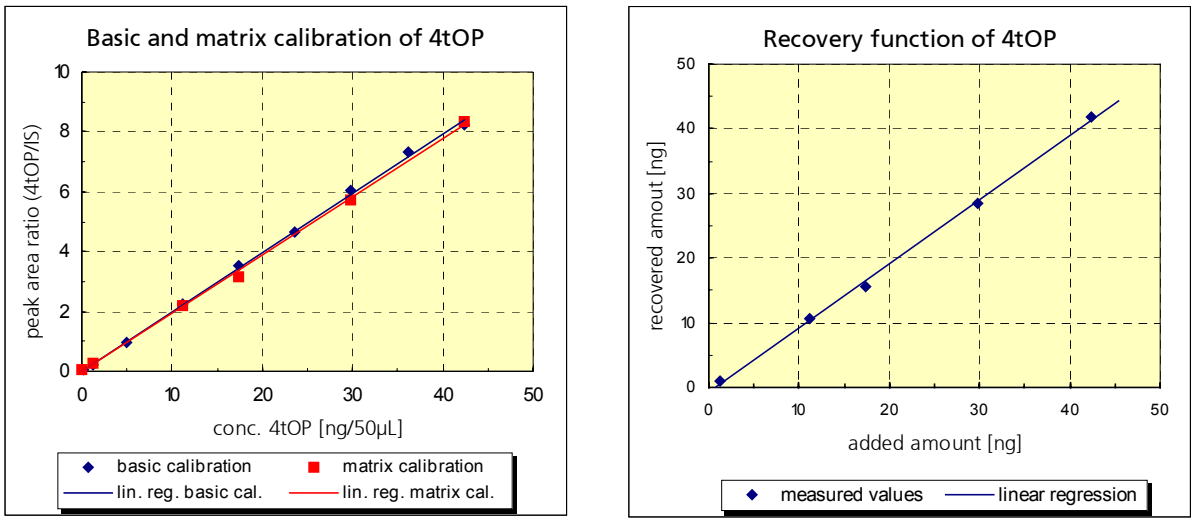
The verification of the recovery function for systematical errors is based on two statistical tests which deliver two statements:

- With a default probability of 99 % no proportional systematical errors is existent when the confidence interval of the slope includes ‘1’
- With a default probability of 99 % no constant systematical errors is existent when the confidence interval of the slope axis intercept includes ‘0’

If the confidence interval of the slope does not include the ‘1’, the analytical method shows a recovery rate which is different from 100 %. The analytical results can then be corrected with this recovery rate. The recovery rate, expressed in percent, is calculated by multiplication of the

slope of the linear recovery function with 100. The calculated recovery rates are listed in Table A 15.

Evaluation of the matrix calibration for the AP2EO resulted in high recovery rates (136 % for 4tOP2EO and 129% for 4NP2EO). The data were obtained on the basis of the appropriate internal standard (IS) 4nNP2EO and correspond to the recovery of the selected IS. Therefore, the matrix calibration was evaluated again with the IS 4nNP1EO. Then the recovery rates for the AP2EO fit 100 %. So the calculation of analytical results for AP2EO was done on the basis of the IS 4nNP1EO.



a) b)

Figure A 6: a) calibration functions of 4tOP
b) recovery functions of 4tOP

A 2.6.5.3 Blanks

Alkylphenols (AP) are mainly used as tensides or as plasticiser or stabiliser in the production of plastics materials. Accordingly, APs are found in nearly all chemicals and solvents used and were sorbed on the surface of the equipment. To minimise the contamination during the sample preparation (and thus the blank values), the following steps were carried out:

- All glass equipment was cleaned carefully and heated up to 250 °C for at least 24 hours; if necessary it was rinsed with organic solvents
- The purity of the silylation agent MSTFA was checked (manufacturer and charge)
- The crimp caps (for closing the GC vials) were heated up to 70 °C for at least 24 hours under a reduced pressure of 50 mbar in a vacuum drying chamber

All these arrangements were not effective to remove all trace amounts of the alkylphenols and alkylphenol ethoxylates sufficiently. Therefore true blank values could not be measured. The values of 4NP and 4NP2EO measured as blanks were above the LOQ (calculated according to DIN 32645). Therefore, a higher LOQ, based on the measured blank values, was derived in the following manner:

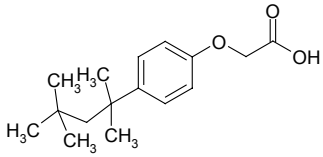
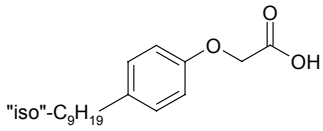
LOQ defined = arithmetic mean of all measured blank values + 3 times the standard deviation.

The new calculated LOQ values are listed in Table A 15, too.

A 2.7 Method: Alkylphenoxy acetic acids

A 2.7.1 Target analytes

Table A 16: Selected Alkylphenoxy acetic acids

Substance		CAS number	Chemical structure	Application/ occurrence
4-*tert.*-Octylphenoxy acetic acid	4tOP1EC	-		transformation product of 4-*tert.*-Octylphenol polyethoxylates
4-Nonylphenoxy acetic acid	4NP1EC	-		transformation product of 4-Nonylphenol polyethoxylates

A 2.7.2 Principle

A solid phase extraction (SPE) method was used for the enrichment of the alkylphenoxy acetic acids from water samples. Initially the samples were acidified with hydrochloric acid to a pH of approx. 2. After addition of the internal standard solution, the analytes were extracted with SPE on Oasis cartridges. The analytes were analytically determined in the extracts by LC/MS/MS.

A 2.7.3 Materials

<u>Internal standard</u>

1H-1H-2H-2H-perfluorooctane sulfonic acid (THPFOS)) was used as internal reference standard (surrogate standard).

Calibration solutions (multicomponent standards)

Calibration solutions were prepared in two different concentration ranges. Spiking volumes for

Range A: 5, 10, 25, 50, 100, 200 ng/L
Range B: 0.05, 0.01, 0.1, 0.25, 0.5, 1 ng/L

Instrumental parameters

HPLC

instrument	HPLC Alliance separation modul 2690/2795, WATERS
autosampler	HPLC Autosampler 2690/2795, WATERS
column	100x2 mm, Betasil C_{18}, KEYSTONE
flow	250 µL/min
injection volume	10 µL
stop time	15 min
column temperature	20 °C
sample temperature	20 °C
solvent A:	methanol +2 mM NH_4Ac (60+40, v/v)
solvent B:	methanol

time (min)	A%	B%
0	100	0
1.0	100	0
4.5	8	92
8.0	8	92
10.0	100	0
15.0	100	0

Mass spectrometry

instrument	LC/MS/MS Quattro Ultima PT, MICROMASS
ionisation	electrospray positive (EI+)

MS/MS parameters

	precursor ion [m/z]	**product ion [m/z]**	**dwell (s) [s]**	**collision energy [eV]**	**cone [V]**
4tOP1EC	262.9	205.1	0.2	16	40
4NP1EC	276.9	219.1	0.2	18	40
THPFOS	427	81.1	0.2	24	40

A 2.7.4 Analytical Procedure

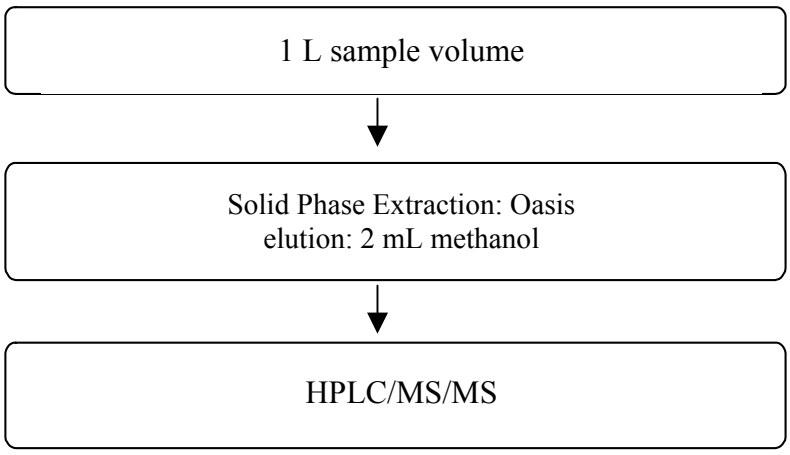


Figure A 7: Analytical scheme: Alkylphenoxy acetic acids

To 1 L of the sample water 100 µL of surrogate standard (THPFOS 5 µg/mL) was added. The pH was checked to be neutral. Then the analytes were extracted by SPE on Oasis HLB cartridges using the following conditions.

- Condition the Oasis SPE cartridges with 10 mL of methanol followed by 5 mL of water (3 drops/s)
- Load the sample (1000 mL)
- Wash with 5 mL of water
- Dry with N_2 for 15 min
- Elute with 2 mL of 100 % methanol
- Dry the cartridge with N_2 for 3 min

Calibration was performed by analyzing the above mentioned calibration solutions before and after each sample series.

A 2.7.5 Quality assurance

On the basis of the calibration results the limits of the analytical method are calculated according to DIN 32645 using a statistical quality assurance program (SQS 98; Perking Elmer). These data are shown in Table A 17.

Table A 17: Calibration and method limit parameters

compound	r^2	RSD %	V_{x0}	LOD ng/L	LOQ ng/L
4tOP1EC	0.9985	5.0	4.8	4.1	15.2
4NP1EC	0.9997	2.9	3.2	2.2	8.1

r correlation coefficient of linear regression
RSD relative standard deviation
V_{x0} relative method standard deviation
LOD Limit of detection
LOQ Limit of quantitation

A 2.8 Yeast estrogen screen (YES) assay

The water samples were prepared using solid phase extraction as described in chapter A 2.3.4.1 for the natural estrogens and contraceptives without clean up. The sample preparations for the chemical and biological analyses were carried out in the same laboratory. For the bioassay the acetone extract was evaporated near dryness and redissolved in 2 ml ethanol. For each sampling point, two water samples were investigated. According to the turbidity of the water, different volumes of water were processed.

The Yeast Estrogen Screen was performed according to Routledge and Sumpter (1996). Graded volumes of the sample extracts or dilutions were added into wells of microtiter plates and the ethanol evaporated. Each concentration was tested in triplicate. 10 µl of ethanol were used as vehicle control. In each plate a concentration-effect curve of 17α-Ethinylestradiol (10^{-8} bis 10^{-12} M) was measured. The test medium containing the yeast cells was added and the plates were incubated for 2 days at 30 °C and shaken twice each day. Galactosidase activity and turbidity were measured at 540 nm and 620 nm, respectively. The colour was corrected for turbidity [Activity = E_{540nm} / E_{620nm}.]. The relative activity of the samples was calculated in relation to the activity of the blanks (activity of the blanks = 1).

Determination of limit of detection and recovery

Based on the concentration-effect curve of the standard substance 17α-Ethinylestradiol the LOD for each test can be indicated. A relative activity above 10 % of the blank value was evaluated to be positive. Relating the LOQ of a test to the highest water equivalents investigated in the respective test, a theoretical LOQ for the water sample can be indicated.

In Table A 18 the processed volumes of the water samples, the highest concentrations of the water equivalents tested and the LOQs expressed in ng/L 17α-Ethinylestradiol equivalents are compiled.

Table A 18: Extracted sample volume, highest concentrations of the water equivalents tested and LOQs of the YES assays.

Water-works	sampling point	sample name	extracted water [L]	water equivalents [L/mL test volume] or conc. EE2 [nM]	LOQ based on EE2 standard curve [ng/L]	LOQ for the respective water sample [ng/L]*
		Blank	-	0		
Waterworks I	raw water	1A	10	1.4	29	0.02
	after ozonation	1B	5.3	2.7	29	0.01
	after GAC	1C	3	6	29	0.01
	after disinfection/ last storage	1D	10	5	29	0.006
	tap water	1E	2	1		
Waterworks II	raw water	2A	2	0.5	12	0.024
	before ozonation	2B	6	1.5	12	0.008
	after ozonation	2C	2	1	12	0.012
	after last drinking water reservoir	2D	10	2.5	12	0.005
	tap water	2E	10	2.5	12	0.005
Waterworks III	raw water	3A	4	1	12	0.015
	after slow sand filtration	3B	10	2.5	12	0.005
	after GAC	3C	10	2.5	12	0.005
	after last disinfection	3D	10	2.5	12	0.005
	tap water	3E	10	2.5	12	0.005
Waterworks IV	raw water	4A	2	1.5	12	0.015
	drinking water after aeration	4D	10	2.5	12	0.015
	tap water	4E	10	2.5	12	0.005

* calculated using LOQ of EE2 and the respective water equivalents in the test.

Determination of recovery

A tap water sample of waterworks I was spiked with a mixture of 17α-Ethinylestradiol, 17β-Estradiol, Estrone, Mestranol and 17β-Estradiol-17-valerate, 5 ng/L each. The sample was extracted as described for the other water samples of the case study. The relative estrogenic potency of the estrogen mixture was calculated as follows:

Table A 19: Relative potencies of steroids in the YES assay.

	ng/L	relative potency in the YES assay	EE2 equivalents [ng/L]
17α-Ethinylestradiol	5	1	5
17β-Estradiol	5	1	5
Estron	5	0.3	1.5
Mestranol	5	0.007	0.035
17β-Estradiol-17-valerate	5	0.1	0.5
EE2 equivalents, theoretical			12.0
EE2 equivalents, in the YES assay			8.5 (71 % of theoretical value)

The estrogenic potencies of the estrogens, except 17β-Estradiol-17-valerate, were results of tests performed at the Fraunhofer Institute (Wenzel et al. 1998). The potency of 17β-Estradiol-17-valerate was assessed with 0.1. The relative activities of the spiked water sample and of EE2 are shown in Figure A 8.

The recovery experiment showed a good consistency of the theoretical concentration and the estrogenic activity determined in the YES assay.

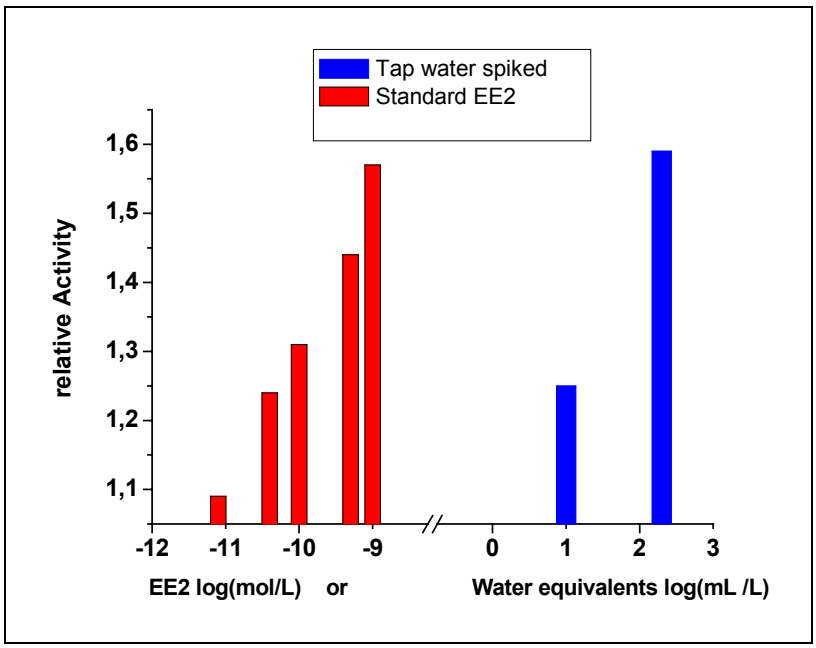


Figure A 8: Determination of recovery in the YES assay.

APPENDIX 3 PARTICIPANTS OF THE NETWORK

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APPENDIX 4 RESULTS OF THE CASE STUDY

Table A 20: Results of the chemical analyses of the water samples of waterworks I

Waterworks I	**LOQ**	**raw water (reservoir water)**		**after ozonation**		**after GAC**		**after chlorination**		**tap water**	
		mean	span	mean	span	mean	span	mean	span	mean	span
Industrial chemicals in ng/L											
Bisphenol A	5.0	6.3	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Phytosteroides in µg/L											
Cholesterol	0.030	0.41	0.34 - 0.48	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Stigmasterol	0.030	0.033	0.030 - 0.036	n.d.	-	n.d.	-	n.d.	-	n.d.	-
β-Sitosterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Cholestan	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Coprostanol	0.030	0.075	0.071 - 0.079	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Campestrol	0.030	0.053	0.044 - 0.062	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Lanosterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Steroid hormones in ng/L											
Estrone	0.50	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17ß-Estradiol	0.10	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Mestranol	0.10	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17a-Ethinylestradiol	0.10	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17ß-Estradiol-17-valerate	0.20	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Estrogenicity in the YES Screen	0.02-0.03	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Contrast media in µg/L											
Iopamidol	0.025	0.42	-	0.36	-	0.28	-	0.24	-	0.18	-
Iopromide	0.010	0.066	-	0.029	-	0.014	-	n.d.	-	n.d.	-
Diatrizoate	0.010	0.11	-	0.12	-	0.15	-	0.15	-	0.10	-
Iothalamic acid	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Ioxithalamic acid	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Iomeprol	0.010	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Iohexol	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-

Table A 20: Results of the chemical analyses of the water samples of waterworks I (continued)

Waterworks I		**LOQ**	**raw water (reservoir water)**		**after ozonation**		**after GAC**		**after chlorination**		**tap water**	
			mean	span	mean	span	mean	span	mean	span	mean	span
Organotin compounds in ng/L organotin cation												
Monomethyltin	MMT	0.70	n.d.	-	n.d.	-	1.1	1.0 - 1.1	n.d.	-	n.d.	-
Dimethyltin	DMT	0.90	1.9	1.8 - 2.0	1.7	1.5 - 1.9	n.d.	-	n.d.	-	n.d.	-
Trimethyltin	TMT	0.90	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Monobutyltin	MBT	1.0	4.1	4.0 - 4.1	2.0	1.9 - 2.1	1.3	1.2 - 1.4	1.5	1.4 - 1.6	1.1	1.0 - 1.1
Dibutyltin	DBT	1.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tributyltin	TBT	1.6	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tetrabutyltin	TTBT	1.1	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Monooctyltin	MOT	1.9	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Dioctyltin	DOT	1.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Alkylphenols in ng/L												
4-t-Octylphenol		0.50	4.1	3.9 - 4.2	1.2	0.9 - 1.5	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol		6.0	59	53 - 65	27	22 - 32	10.1	7.1 - 13.0	n.d.	-	n.d.	-
Alkylphenol ethoxylates in ng/L												
4-t-Octylphenol monoethoxylate		0.50	0.9	0.8 - 1.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-t-Octylphenol diethoxylate		0.50	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol monoethoxylate		2.5	10.0	8.9 - 11.0	3.9	3.7 - 4.1	n.d.	-	3.2	2.7 - 3.6	2.6	2.5 - 2.6
4-Nonylphenol diethoxylate		3.0	12	11 - 13	4.4	3.5 - 5.3	n.d.	-	4.5	3.1 - 5.8	n.d.	-
Alkylphenoxy acetic acids in ng/L												
4-*tert.*-Octylphenoxy acetic acid		15	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenoxy acetic acid		8.0	162	155 - 168	30	29 - 30	n.d.	-	n.d.	-	n.d.	-

LOQ = Limit of Quantitation
n.d. = below LOQ

Table A 21: Results of the chemical analyses of the water samples of waterworks II

Waterworks II	**LOQ**	**raw water A (surface water)**		**raw water B (surface water)**		**after ozonation**		**after slow sand filter**		**tap water**	
		mean	span	mean	span	mean	span	mean	span	mean	span
Industrial chemicals in ng/L											
Bisphenol A	5.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Phytosteroides in µg/L											
Cholesterol	0.030	0.032	0.024 - 0.040	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Stigmasterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
β-Sitosterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Cholestan	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Coprostanol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Campestrol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Lanosterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Steroid hormones in ng/L											
Estrone	0.50	3.0	2.5 - 3.4	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17ß-Estradiol	0.10	0.38	0.30 - 0.45	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Mestranol	0.10	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17a-Ethinylestradiol	0.10	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17β-Estradiol-17-valerate	0.20	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Estrogenicity in the YES Screen ng/L	0.012-0.045	0.06	0.06	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Contrast media in µg/L											
Iopamidol	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Iopromide	0.010	n.d.	-	0.014	-	n.d.	-	n.d.	-	n.d.	-
Diatrizoate	0.010	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Iothalamic acid	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Ioxithalamic acid	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Iomeprol	0.010	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Iohexol	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-

Table A 21: Results of the chemical analyses of the water samples of waterworks II (continued)

Waterworks II		**LOQ**	**raw water A (surface water)**		**raw water B (surface water)**		**after ozonation**		**after slow sand filter**		**tap water**	
			mean	span	mean	span	mean	span	mean	span	mean	span
Organotin compounds in ng/L organotin cation												
Monomethyltin	MMT	0.7	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Dimethyltin	DMT	0.9	n.d.	-	2.3	2.2 - 2.4	2.1	2.0 - 2.1	n.d.	-	n.d.	-
Trimethyltin	TMT	0.9	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Monobutyltin	MBT	1.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Dibutyltin	DBT	1.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tributyltin	TBT	1.6	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tetrabutyltin	TTBT	1.1	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Monooctyltin	MOT	1.9	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Dioctyltin	DOT	1.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Alkylphenols in ng/L												
4-t-Octylphenol		0.5	1.6	1.4 - 1.8	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol		6.0	21	17 - 25	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Alkylphenol ethoxylates in ng/L												
4-t-Octylphenol monoethoxylate		0.5	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-t-Octylphenol diethoxylate		0.5	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol monoethoxylate		2.5	12	11 - 12	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol diethoxylate		3.0	7.1	6.2 - 7.9	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Alkylphenoxy acetic acids in ng/L												
4-*tert.*-Octylphenoxy acetic acid		15.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenoxy acetic acid		8.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-

LOQ = Limit of Quantitation
n.d. = below LOQ

Table A 22: Results of the chemical analyses of the water samples of waterworks III

Waterworks III	**LOQ**	**raw water (canal water)**		**after slow sand filter**		**after GAC**		**after chlorination**		**tap water**	
		mean	span	mean	span	mean	span	mean	span	mean	span
Industrial chemicals in ng/L											
Bisphenol A	5.0	6.1	5.1 - 7.0	< 5,0	-	n.d.	-	n.d.	-	n.d.	-
Phytosteroides in µg/L											
Cholesterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Stigmasterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	0.051	0.038 - 0.063
β-Sitosterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	0.14	0.099 - 0.179
Cholestan	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Coprostanol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Campestrol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Lanosterol	0.030	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Steroid hormones in ng/L											
Estrone	0.50	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17ß-Estradiol	0.10	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Mestranol	0.10	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17a-Ethinylestradiol	0.10	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
17β-Estradiol-17-valerate	0.20	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Estrogenicity in the YES Screen	0.005-0.03	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Contrast media in µg/L											
Iopamidol	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Iopromide	0.010	0.21	-	0.012	-	n.d.	-	0.020	-	0.029	-
Diatrizoate	0.010	0.073	-	0.031	-	0.041	-	0.042	-	0.045	-
Iothalamic acid	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Ioxithalamic acid	0.025	0.037	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Iomeprol	0.010	0.041	-	n.d.	-	n.d.	-	0.010	-	0.012	-
Iohexol	0.025	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-

Table A 22: Results of the chemical analyses of the water samples of waterworks III (continued)

Waterworks III		**LOQ**	**raw water (canal water)**		**after slow sand filter**		**after GAC**		**after chlorination**		**tap water**	
			mean	span	mean	span	mean	span	mean	span	mean	span
Organotin compounds in ng/L organotin cation												
Monomethyltin	MMT	0.7	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Dimethyltin	DMT	0.9	3.6	3.4 - 3.7	2.9	2.7 - 3.0	n.d.	-	n.d.	-	n.d.	-
Trimethyltin	TMT	0.9	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Monobutyltin	MBT	1.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Dibutyltin	DBT	1.0	1.6	1.4 - 1.8	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tributyltin	TBT	1.6	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tetrabutyltin	TTBT	1.1	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Monooctyltin	MOT	1.9	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Dioctyltin	DOT	1.0	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Alkylphenols in ng/L												
4-t-Octylphenol		0.5	1.2	1.0 - 1.3	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol		4.0	31	28 - 33	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Alkylphenol ethoxylates in ng/L												
4-t-Octylphenol monoethoxylate		0.5	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-t-Octylphenol diethoxylate		0.5	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol monoethoxylate		2.5	7.2	5.5 - 8.9	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol diethoxylate		1.5	12	9.7 - 15	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Alkylphenoxy acetic acids in ng/L												
4-*tert.*-Octylphenoxy acetic acid		15	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenoxy acetic acid		8.0	94	88 - 99	n.d.	-	n.d.	-	n.d.	-	11	11 - 12

LOQ = Limit of Quantitation
n.d. = below LOQ

Table A 23: Results of the chemical analyses of the water samples of waterworks IV

Waterworks IV	**LOQ**	**raw water (deep groundwater)**		**drinking water after aeration**		**tap water**	
		mean	span	mean	span	mean	span
Industrial chemicals in ng/L							
Bisphenol A	5.0	n.d.	-	n.d.	-	n.d.	-
Phytosteroides in µg/L							
Cholesterol	0.030	n.d.	-	n.d.	-	n.d.	-
Stigmasterol	0.030	n.d.	-	n.d.	-	n.d.	-
β-Sitosterol	0.030	n.d.	-	n.d.	-	n.d.	-
Cholestan	0.030	n.d.	-	n.d.	-	n.d.	-
Coprostanol	0.030	n.d.	-	n.d.	-	n.d.	-
Campestrol	0.030	n.d.	-	n.d.	-	n.d.	-
Lanosterol	0.030	n.d.	-	n.d.	-	n.d.	-
Steroid hormones in ng/L							
Estrone	0.50	n.d.	-	n.d.	-	n.d.	-
17ß-Estradiol	0.10	n.d.	-	n.d.	-	n.d.	-
Mestranol	0.10	n.d.	-	n.d.	-	n.d.	-
17a-Ethinylestradiol	0.10	n.d.	-	n.d.	-	n.d.	-
17β-Estradiol-17-valerate	0.20	n.d.	-	n.d.	-	n.d.	-
Estrogenicity in the YES Screen	0.005-0.03	n.d.	-	n.d.	-	n.d.	-
Contrast media in µg/L							
Iopamidol	0.025	n.d.	-	n.d.	-	n.d.	-
Iopromide	0.010	n.d.	-	n.d.	-	n.d.	-
Diatrizoate	0.010	0.019	-	0.014	-	0.018	-
Iothalamic acid	0.025	n.d.	-	n.d.	-	n.d.	-
Ioxithalamic acid	0.025	n.d.	-	n.d.	-	n.d.	-
Iomeprol	0.010	n.d.	-	n.d.	-	n.d.	-
Iohexol	0.025	n.d.	-	n.d.	-	n.d.	-

Table A 23: Results of the chemical analyses of the water samples of Waterworks IV (continued)

Waterworks IV		**LOQ**	**raw water (deep groundwater)**		**drinking water after aeration**		**tap water**	
			mean	span	mean	span	mean	span
Organotin compounds in ng/L organotin cation								
Monomethyltin	MMT	0.7	n.d.	-	n.d.	-	n.d.	-
Dimethyltin	DMT	0.9	n.d.	-	n.d.	-	n.d.	-
Trimethyltin	TMT	0.9	n.d.	-	n.d.	-	n.d.	-
Monobutyltin	MBT	1.0	n.d.	-	n.d.	-	n.d.	-
Dibutyltin	DBT	1.0	n.d.	-	n.d.	-	n.d.	-
Tributyltin	TBT	1.6	n.d.	-	n.d.	-	n.d.	-
Tetrabutyltin	TTBT	1.1	n.d.	-	n.d.	-	n.d.	-
Monooctyltin	MOT	1.9	n.d.	-	n.d.	-	n.d.	-
Dioctyltin	DOT	1.0	n.d.	-	n.d.	-	n.d.	-
Alkylphenols in ng/L								
4-t-Octylphenol		0.5	n.d.	-	0.7	0.6 - 0.7	n.d.	-
4-Nonylphenol		4.0	n.d.	-	7.9	7.9	n.d.	-
Alkylphenol ethoxylates in ng/L								
4-t-Octylphenol monoethoxylate		0.5	n.d.	-	n.d.	-	n.d.	-
4-t-Octylphenol diethoxylate		0.5	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol monoethoxylate		2.5	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenol diethoxylate		1.5	n.d.	-	n.d.	-	n.d.	-
Alkylphenoxy acetic acids in ng/L								
4-*tert.*-Octylphenoxy acetic acid		15	n.d.	-	n.d.	-	n.d.	-
4-Nonylphenoxy acetic acid		8.0	n.d.	-	n.d.	-	n.d.	-

LOQ = Limit of Quantitation
n.d. = below LOQ

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LIST OF ABBREVIATIONS

4NP	4-Nonylphenol
4NP1EO	4-Nonylphenol monoethoxylate
4NP2EC	4-Nonylphenoxy acetic acid
4NP2EO	4-Nonylphenol diethoxylate
4tOP	4-*tert*.-Octylphenol
4tOP1EO	4-*tert*.-Octylphenol monoethoxylate
4tOP2EC	4-*tert*.-Octylphenoxy acetic acid
4tOP2EO	4-*tert*.-Octylphenol diethoxylate
a	intercept of regression line
ADI	acceptable daily intake (commonly used for substances intentionally released into the environment)
AGC	automatic gain control
amu	atom mass units
AP	Alkylphenols
AP1EO	Alkylphenol monoethoxylates
AP2EO	Alkylphenol diethoxylates
APxEO	AP, AP1EO and AP2EO
b	slope of regression line
bw	body weight
BGVV	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (formerly). New name: Federal Institute for Risk Assessment
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
DBT	Dibutyltin
DCA	Dichloroaniline
DMI	Desmethoxy iopromide
DMT	Dimethyltin
DOT	Dioctyltin
DTE	Dithioerytrol
EDC	Endocrine disrupting chemicals
EDS	Endocrine disrupting substances
EI	electron impact
EU	European Union
EUREAU	European Union of National Associates of Water Suppliers and Waste Water Services
GAC	granulated active charcoal
GC	Gaschromatography
GC/AED	Gaschromatography with atomic emission detection
GC/MS	Gaschromatography with mass spectrometric detection

GC/MS/MS	Gaschromatography with tandem mass spectrometric detection
HPLC	High Performance Liquid Chromatography
HPLC/MS	High Performance Liquid Chromatography with mass spectrometric detection
IME	Fraunhofer Institute of Molecular Biology and Applied Ecology
IS	internal standard
KAS	cold injection system (Kaltaufgabe System)
kg	kilogram
LC	Liquid Chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
MBT	Monobutyltin
MMT	Monomethyltin
mmu	milli mass units
MOT	Monooctyltin
MPS	multi purpose sampler
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MSTFA	*N*-Methyl-*N*-trimethylsilyltrifluoracetamide
NOAEL	No observed adverse effect level
n.d.	not determined(value below LOQ)
OSPAR	Oslo Paris Convention: The Convention for the Protection of the Marine Environment of the Nort East Atlantic.
OTC	Organotin cation
PIT	prescan ionisation time
POP	persistent organic pollutants
r	correlation coefficient of linear regression
rel.	relative
RSD	relative standard deviation
SCF	Scientific Committee of Food
SGE	distributor for chromatographic products
SPE	solid phase extraction
TBT	Tributyltin
TDI	tolerable daily intake (commonly used for substances unintentionally released into the environment)
TMSI	Trimethylsilylimidazole
TMT	Trimethyltin
TSH	Thyroid stimulating hormone
TTBT	Tetrabutyltin
TW	Tap water (site Schmallenberg, Germany)
WHO	World Health Organisation
WW	Waterworks