ASSOCIATION OF RIVER WATERWORKS

RIWA

November 2000

Endocrine disrupting compounds in the Rhine and Meuse basin

Occurrence in surface, process and drinking water

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Sub-project of the National Research Project on the Occurrence of Endocrine Disrupting Compounds

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Publisher

Association of River Waterworks - RIWA

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1.1 Summary

The presence of anthropogenic substances which are able to influence the endocrine systems of organisms living in an aquatic environment is causing serious concern among both authorities and scientists. And as a result of media attention, the consumer has also been warned about the possibility of contamination of humans via drinking water.

A clear relationship has been found between the presence of endocrine- or hormone-disrupting substances and developmental changes in a number of animal species. As early as the 1980s, research by Reijnders (Rei 1985) showed that the reproductive success of seal populations in the North Sea was affected by the presence of PCBs in the water. In the Scheldt estuary, abnormalities were found in sea slugs, which showed imposex features[1]. It was proved that there was a relation between the concentrations of the biocide tributyltin and the abnormalities found in the animals. These and many other examples in international waters indicate that the release of substances with endocrine effects, particularly on those hormonal systems which are responsible for sexual and reproductive development, constitute a threat to wildlife. It seems entirely probable that sooner or later a relation will be found between such discharges and undesirable effects on humans. Suggestions to that effect have frequently been made, and although there is currently no conclusive evidence of such negative effects on humans, currently available evidence cannot exclude this possibility either.

In the early 1990s, the presence of endocrine-disrupting substances in the environment was linked to the trend of decreasing sperm counts found in men over the last 50 years. This was assumed to be caused by the presence of substances which might affect human hormonal reproductive systems. It is undeniable that humans are currently more intensively exposed to such substances than in the past. The huge numbers of chemicals being used on a daily basis, some of which are known to produce endocrine effects, make this abundantly clear. This does not, however, necessarily mean that an immediate effect is to be expected. Before the substances can reach the receptors which are sensitive to them, they have to overcome many barriers. Bioavailability of such substances in the cell is not a simple matter. Before substances can reach the cell in a form which allows them to produce an effect, they have to endure the body's metabolism and have to pass through the cell wall. In addition, concentrations are usually very low and exposure times very short.

Nevertheless, there may well be situations in which humans are extremely vulnerable, for instance during embryonic and foetal development, or in situations of accelerated release of lipophilic, hormone-disrupting substances stored in e.g. fatty tissue. Thus, we cannot afford to ignore the potential effects of these substances.

Some years ago, a number of national organizations in the Netherlands which are responsible for the quality of surface waters in river basins and coastal areas decided to start a joint research programme to investigate the problem of hormone-disrupting substances. For this purpose, they set up a joint project to survey the presence of these substances in surface waters in the basins of the rivers Rhine and Meuse.

The present report publishes RIWA's contribution to this major research effort, comprising the studies funded by RIWA. The sampling points include locations in the Rhine and Meuse basins, as well as locations at treatment plants used by Dutch water supply companies. The latter sampling points may concern various stages of the purification process, as well as the finished product, i.e., drinking water.

The aim of the RIWA subproject entitled 'Surface, process and drinking water' is to assess the potential endocrine effects of these substances in the types of water referred to in

[1] Imposex is the development of masculine primary sexual characteristics in female gastropods, with the effect that reproduction is disturbed. The original female characteristics remain intact.

the name, in order to evaluate the potential risks for the production of drinking water. In addition, a number of substances are being assayed which are known to have potential pseudo-endocrine effects and which are present in the environment. Subsequently, attempts will be made to assess which of the oestrogenic effects found can be attributed to one or more individual substances.

Sampling took place in three rounds, in March, June and September of 1999, at a large number of locations along the rivers Meuse and Rhine in the Netherlands, Belgium and Germany. The sampling locations were selected on the basis of RIWA's existing sampling network. The Dutch water companies participating in RIWA were free to choose their own sampling points and were allowed to change the points after each sampling round, as and if they saw fit to do so. The samples were distributed over a number of laboratories for preparation and analysis.

The bio assay results show that only in a single sample oestrogenic activity was found in drinking water in any of the three sampling rounds. The chemical analyses of oestrogenic substances confirmed this finding: very few samples tested positive, and these invariably concerned extremely low concentrations. Similarly, only a few of the process water samples showed any oestrogenic activity, with the occasional sample showing concentrations above the detection limit.

By contrast, the measurements on river water showed that increased oestrogenic activity in a number of places. The sampling locations were highly diverse, ranging from extraction points of water supply companies to plumes of sewage treatment plants and industrial wastewater plumes. The analyses show considerable fluctuations in the concentrations of the substances analysed. Statistical tests showed a significant difference in oestrogenic potential between the Meuse and the Rhine, with the Meuse on average showing twice the activity levels of the Rhine. On the whole, oestrogenic activity was found to be highest near the end of winter, which is remarkable since this is also the period with the highest discharge levels in these rivers.

Analysis of the samples from individual water supply companies showed that their water purification methods were consistently adequate to remove hormone-disruptive substances from drinking water. The measurements showed pre-purification in basins to be more effective than extraction by means of riverbank infiltration.

The project design involved simultaneous sampling at various locations. It must be emphasized that this method means that it cannot be decided whether a substance found to be present in high concentrations at a particular extraction point is actually removed during the purification process. It is therefore recommended to increase the sampling frequency along the rivers and to continue monitoring over a longer period. This would provide information on fluctuations in the concentrations of the various substances. The number of sampling locations could be substantially reduced, to about two per river. In addition, it is recommended to investigate more closely the water purification process at those water supply companies which are at increased risk.

Since the spectrum of hormone-disrupting substances is changeable, and since not all of these substances were detected in the present study, it is recommended that the selection of substances to be monitored should be reviewed. The results of the chemical analysis need to be supported by both a bio assay test (e.g., ERC test) and a vitellogenin test, using rainbow trout in situ.

1.2 Samenvatting

De aanwezigheid van antropogene stoffen die endocriene systemen van organismen die in het aquatisch milieu leven kunnen beïnvloeden is een zorg die bij de overheid en bij wetenschappers duidelijk leeft. Inmiddels is door de aandacht in de media ook de consument gewaarschuwd voor de mogelijke contaminatie van de mens via het drinkwater.

Er is een duidelijk verband aangetoond tussen de aanwezigheid van endocrien- of hormoon ontregelende stoffen en de wijze waarop een aantal dieren zich ontwikkelt. Al in de jaren tachtig toonde onderzoek van Reijnders (Rei 1985) aan dat de voortplanting van zeehonden in de Noordzee werd gehinderd door de aanwezigheid van PCB's in het water. Ook in het estuarium van de Schelde werden er afwijkingen gevonden bij de zeeslakjes, die imposex[2] vertoonden. Er werd aangetoond dat er een verband bestond tussen de concentraties van het biocide middel tributyltin en de afwijking in deze dieren.

Deze en veel andere voorbeelden in internationale wateren geven aan dat de lozing van stoffen met een endocriene werking op met name de hormoonsystemen die zorgdragen voor de geslachts- en voortplantingontwikkeling, een bedreiging vormt voor in het wild levende dieren. Het laat zich moeilijk raden dat er vroeg of laat een verband zal worden gevonden tussen de lozingen en ongewenste effecten bij de mens. Suggesties in die richting worden veelvuldig gedaan en alhoewel er op dit moment geen sluitend bewijs is dat er een negatief effect is voor de mens, is dit met de thans beschikbare gegevens niet uit te sluiten.

Begin jaren negentig werd de aanwezigheid van endocrien ontregelende stoffen in het milieu in verband gebracht met de trendmatige afname van het aantal spermacellen bij de man gedurende laatste 50 jaar. De oorzaak werd gezocht bij de aanwezigheid van stoffen die een effect hebben op de hormonale voortplantingssystemen van de mens. Het is onmiskenbaar waar dat de huidige mens meer dan vroeger blootstaat aan deze stoffen. De grote hoeveelheid chemische stoffen die dagelijks gebruikt worden en waarvan bekend is dat een aantal hiervan een endocriene werking heeft, is daar het bewijs van. Hiermee is niet gezegd dat er een direct effect kan worden verwacht. Voordat de receptoren die gevoelig zijn voor de werking van de stoffen worden bereikt, zullen er vele barrières geslecht moeten worden. De biobeschikbaarheid in de cel van de stoffen is geen eenduidige aangelegenheid. Voordat de stoffen de cel kunnen bereiken in een vorm die geschikt is voor het geven van een effect, zal een stof onderworpen worden aan het lichaamsmetabolisme en moet een stof de celwand passeren. Daar komt bij dat de concentraties en de duur van de blootstelling zeer laag zijn. Niettemin houdt men rekening met situaties waarin de mens zeer kwetsbaar is, te weten tijdens de embryonale en foetale ontwikkeling, of in omstandigheden waarin de lipofiele hormoon ontregelende stoffen die opgeslagen zijn in bij voorbeeld vetweefsel versneld vrijkomen. Dit is de reden waarom er niet zomaar voorbij gegaan kan worden aan de potentiële invloed van deze stoffen.

Een aantal nationale instanties, verantwoordelijk voor de kwaliteit van oppervlaktewater in rivier- en kustgebieden hebben enige jaren geleden besloten het probleem van de hormoon ontregelende stoffen gezamenlijk te onderzoeken. Hiertoe is een project opgezet voor het inventariseren van de aanwezigheid van deze stoffen in oppervlaktewateren in het Maas en Rijn stroomgebied..

In dit rapport wordt het RIWA aandeel van dit grote onderzoek gepubliceerd. Het gaat om de onderzoeken die gefinancierd zijn door de RIWA-organisatie. De meetpunten omvatten

[2] Imposex is de ontwikkeling van manlijke primaire sexuele karakteristieken in vrouwlijke gastropoden op zodanige wijze dat de voortplanting wordt verstoord. De oorspronkelijke vrouwlijke karacteristieken blijven in takt.

locaties in het Rijn- en Maas stroomgebied, en locaties bij de waterleiding-bedrijven in Nederland. Deze laatste meetpunten kunnen punten zijn op verschillende fasen in het zuiveringsproces maar ook drinkwater, het eindproduct

De doelstelling van het RIWA deelproject 'Oppervlakte-, proces- en drinkwater zoet' is om in deze watersoorten stoffen met mogelijke endocriene effecten te bepalen. Dit alles met als doel om een inschatting te maken van de eventuele risiko's voor de drinkwaterproduktie. Bovendien worden er een aantal stoffen gemeten waarvan vast staat dat ze een pseudo-endocrien effect kunnen geven en dat ze in het milieu aangetroffen kunnen worden. Na meting wordt vastgesteld in welke mate het gemeten oestrogene effect is toe te wijzen aan de een of meer individuele stoffen.

De monstername heeft in 1999 plaats gevonden op drie tijdstippen, namelijk in maart, juni en september op tal van locaties langs de Maas en de Rijn in Nederland, België en Duitsland. De monsterlocaties zijn gekozen op basis van het RIWA meetnet. De aangesloten RIWA-bedrijven in Nederland zijn vrij geweest in de keuze van de eigen monsterpunten en konden na iedere meetronde op basis van nieuwe inzichten de monstername aanpassen. De monsters zijn gedistribueerd over de verschillende laboratoria voor de opwerking van de watermonsters en de analyse.

De resultaten van de bioassay laten zien dat in de drinkwatermonsters slechts in een enkel geval in een van de meetrondes oestrogene activiteit is gevonden. De chemische analyses van de oestrogene stoffen bevestigen dit: slechts zeer sporadisch is er een positief monster en in die gevallen ging het om een zeer lage concentratie. De proceswatermonsters vertonen eveneens in een enkel geval enige oestrogene activiteit. Er wordt af en toe een gehalte boven de detectielimiet gevonden.

De metingen in de rivieren tonen echter aan dat er op een aantal plaatsen sprake is van een verhoogde oestrogene activiteit. De monsternamelocaties zijn erg divers en omvatten innamepunten van waterleidingbedrijven, pluimen van rioolwaterzuiveringsinstallaties en pluimen van afvalwater van industrieën. De analyses tonen aan dat er sterke fluctuaties kunnen optreden in de concentraties van de onderzochte stoffen. Na het verrichten van statistische tests blijkt dat er een significant verschil is tussen de oestrogene potentie van de Maas en de Rijn. De Maas blijkt een gemiddeld twee keer zo grote activiteit te hebben. Het blijkt dat over het geheel genomen de oestrogene activiteit aan het einde van de winter het grootst is. Dit is extra opmerkelijk omdat het debiet van de rivieren in die periode eveneens het grootst is.

In het geval van de individuele waterleidingbedrijven blijkt dat de waterzuivering in alle gevallen afdoende werkt bij de bereiding van drinkwater als het gaat om hormoon ontregelende stoffen. De voorzuivering d.m.v. een bekken blijkt op grond van de verrichte metingen effectiever dan de winning via oeverfiltratie.

Bij de opzet van dit project is gekozen voor een monstername op hetzelfde tijdstip op de verschillende locaties. Hierbij dient te worden aangemerkt dat door deze wijze van gelijktijdige monstername niet valt te concluderen of een stof die bij het innamepunt in een hoge concentratie aanwezig is ook daadwerkelijk door de zuivering wordt verwijderd. Om die reden wordt het aanbevolen om de monstername frequentie in de rivieren te verhogen en gedurende langere tijd te monitoren. Op deze manier wordt inzicht verkregen in de mate waarin de concentraties van de verschillende stoffen varieert. Het aantal monstername locaties kan aanmerkelijk worden teruggebracht tot ongeveer twee per rivier. Daarnaast wordt aanbevolen om bij bedrijven die een verhoogd risico lopen het waterzuiveringsproces verder te onderzoeken.

Aangezien het scala van hormoon ontregelende stoffen aan verandering onderhevig is en omdat niet alle stoffen in dit project werden aangetroffen, wordt aanbevolen om de keuze van de te analyseren stoffen opnieuw onder de loep te nemen.

De chemische analyses dienen te worden ondersteund met zowel een bioassay test (b.v. ERC test) als een vitellogenine test met de regenboog forel op locatie.

2. Introduction

In the early 1970s, researchers reported a possible relation between the occurrence of environmental contaminants and the decline in the reproduction of colonies of sea gulls on the Channel Islands in California (Sch 1970). These studies confirmed that organochlorine pesticides, particularly DDT, were responsible for this effect (Sow 1980).
Similar observations have been made in Dutch coastal and estuarine waters. Colonies of common terns have been studied, and the birds were found to be highly polluted with polychlorinatedbiphenyls (PCBs), showing effects on their endocrine systems (Bos 1995). Furthermore, researchers have demonstrated that the presence of PCBs was responsible for the severely decreased population size of the common seal (Rei 1985).

Another finding was the development of imposex in female specimens of the common whelk (Bla 1970) and the dog-whelk (Men 1996), caused by the presence of organotin compounds in the estuarine waters of Meuse and Rhine. These substances are used as fungicides in agriculture and as anti-fouling compounds for boats; they stimulate female whelks to develop masculine primary characteristics, which obstructs reproduction. It is expected that these species will eventually become extinct in this area.

These and many other examples in open waters all over the world have one common factor: pollutants in the environment apparently have some detrimental effect on the reproductive system of aquatic species. Substances which have been identified as having this effect are called Endocrine Disrupting Compounds (EDCs). Such substances have an adverse or stimulating effect on the hormonal system in a species and are likely to influence a variety of hormonal systems. Systems for which there is proof of these effects are the thyroid and the reproductive hormonal system.

The hormones regulating the reproductive system are called oestrogenic hormones. These comprise the compounds 17β-oestradiol, oestron and 17α-oestradiol, endogenous hormones which have a function in the female reproductive system. Compounds with these or similar steroid structures are found in relatively large quantities in females of many animal species, including human beings.

Exogenous chemical compounds which exhibit an effect on the reproductive hormonal system are called xeno-oestrogens. This group comprises a growing number of compounds, the best-known representatives of which are the Organo Chlorine Pesticides (OCPs), the Poly Chlorinated Biphenyls (PCBs), dioxines and the chlorotriazine pesticides. Other, and relatively new groups which have been identified as potentially oestrogenic are: the alkylphenolpolyethoxylates, the phthalates, the polybromobiphenyls, the polybromodiphenylethers and the compound bisphenol A.

Since compounds have been detected in bacteria with hormonal functions and structures related to those in humans, it is plausible that man will eventually be affected by the huge concentrations of oestrogen-mimicking compounds which are spreading throughout the environment (Sha 1998). In 1993, an article about the decline of the male fertility, expressed as the percentage of healthy sperm cells and the motility of sperm cells and based on a literature study (Car 1993), caused alarm among the general public. Many have criticized the study and no satisfactory consensus could be established (Ler 1995; Ols 1995). However, the impact of this article has mobilized authorities to take action such as the screening of chemical compounds for endocrine effects (Col 1998) and assessment studies of xeno-oestrogenic compounds in the environment. One such project is the LOES project.

The LOES project
In 1998, a group of institutes in the Netherlands started a joint project called the Landelijk Onderzoek Oestrogene Stoffen (LOES) or Dutch National Investigation on Estrogenic Compounds in the Aquatic Environment. The project is being carried out in the Dutch delta of

the rivers Meuse and Rhine. Samples are taken from various compartments, such as surface water, sediments and biota. The goal of the project is to determine the endocrine effect on fish populations of the occurrence of xeno-oestrogens in water systems. This effect will be studied by means of in vivo experiments with fish within the scope of the LOES project. In addition, the concentrations of these xeno-oestrogens and their endocrine effects will be determined in a relatively large number of samples, using in-vitro bio assays. The combination of these techniques will presumably provide an answer to the question what compounds are responsible for which part of the endocrine effect.

RIWA (Association of River Waterworks, Amsterdam) is one of the institutes that have commissioned the LOES project and represents the Dutch drinking water companies which produce their potable water from river water. The area in which water samples are being taken has been extended to the neighbouring countries in the Meuse and Rhine basin.

The RIWA sub-project

This report describes the results of the analysis of the RIWA samples and the interpretation of these results. The water samples were taken from surface, process and drinking water which are of interest to the Dutch water companies along the Rhine and Meuse. The study also involved the surface water samples from Meuse and Rhine which were taken at locations in Belgium and Germany.

The goal of the study was to determine the concentration of a selected number of natural and synthetic endocrine disrupting compounds in various water matrices and to determine the oestrogenic effect of the samples by bio assay methods. Moreover it was studied whether and to what extend the oestrogenic effect measured by the bio assays could be related to the measured concentrations of individual compounds.

The samples were taken in three separate rounds: one in March, one in June and one in September. The samples were collected at the sampling locations which are regularly used in RIWA's routine monitoring programmes and, in addition, at sampling points identified by the participating drinking water companies. They were transported to the various laboratories for sample preparation and analysis. The data were centrally collected by RIZA (Institute for Inland Water Management and Waste Water Treatment) in a results database.

The compounds examined in the present study and reported here are the hormones 17α- and 17β-oestradiol, oestron, and 17β-ethinyloestradiol, as well as a set of industrial substances, i.e. bisphenol A, a number of phthalates and some alkylphenolpoly-ethoxylates and their degradation products. The ERC assay was applied as the bio assay study. Some general parameters were also determined: chlorine content, salinity, particulate matter content and ashes remaining after evaporation and burning.

The present report analyses the resulting data in such a way that a preliminary assessment of the occurrence of endocrine disrupting compounds can be made. It provides a clear overview of the concentrations at the various locations during the three sampling rounds. We wanted to determine whether there were any locations showing recurrent higher levels of contamination. This information should allow us to identify locations with a higher risk.

Plots of concentration versus location provide information about the situation along the two rivers. What for instance is the relation between the samples collected on different dates and the mean concentration for each location along the two rivers. Concentrations might be seasonal and there might be a difference in levels between Rhine and Meuse.

The situation at the extraction points of the water companies was studied, as well as the performance of their purification systems regarding the elimination of the compounds. Is the way the water companies purify river water to produce drinking water adequate to supply safe drinking water and what are the differences between these processes?

The value of the bio assay test was investigated as well as the relation between the test and the concentrations of the individual endocrine disruptors. Furthermore, the possible risk of the production of drinking water containing endocrine disrupting substances was evaluated.

At the European workshop on the Impact of Endocrine Disruptors on Human Health and Wildlife in 1997 (Eur 1997) the term endocrine disruptor was defined as:
"An exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function".

3. Physiological significance of oestrogenic hormones and the consequences for organisms of exposition to xeno-oestrogens

Many aspects of the physiological functions are controlled by the endocrine system. It helps to maintain the internal environment of an organism in a state of homeostasis, and enables it to alter in response to changes in the external environment. The endocrine system functions by means of glands which secrete chemicals or hormones. The term endocrine refers to glands that release their products into the extra cellular compartment and thence into the bloodstream (Gre 1994). The oestrogenic hormones belong to the group of sex hormones. The glands, the ovaries in females and the testes in males, release the hormones into the bloodstream, after which they are transported to cells or organs at distant sites. The chemical structures of the oestrogenic hormones are of the steroid type. These steroids are synthesized in the body from cholesterol and possess lipophilic (fat-soluble) properties. Lipophilic molecules can diffuse freely across all biological membranes.

Fig 3.1 shows the mechanism of action of oestrogens. After the hormone is released into the bloodstream, the sex hormones are bound specifically and with high affinity by the protein SHBG (Sex Hormone Binding Protein). There is an equilibrium between free and SHBG-bound hormone, so that a fixed proportion of the hormone travels free. It is currently believed that only the free fraction of the hormone is physiologically active on the target cells; while the bound fraction is inactive. The steroids can easily diffuse through the cell membrane and the membrane of the cell nucleus.

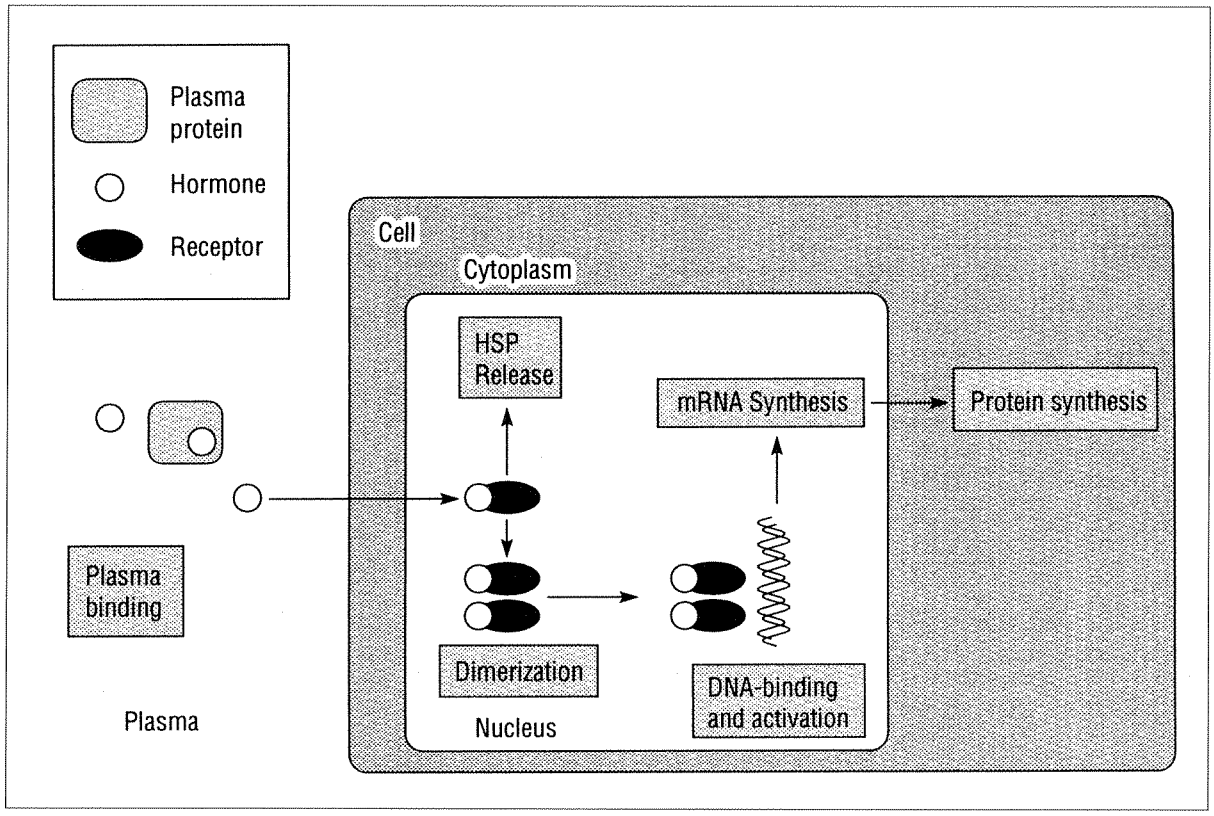

Fig. 3.1 The mechanism of action of oestrogenic hormones.

The free oestrogenic hormones combine with specific receptor proteins which are located in the cell nucleus. These receptors are called oestrogenic receptors (ER), and the hormones act via these receptors to initiate DNA transcription. As a result of hormone binding, the receptor dissociates from a heat shock protein (HSP) with which it is associated. Two molecules of the receptor dimerize and bind to the DNA at specific hormone response elements (HRE), and then initiate transcription via mRNA synthesis and subsequent protein synthesis.

Receptor antagonism

Antagonism of hormone-receptor interactions is a very important aspect in endocrinology, not only in terms of the study of hormone-receptor interaction, but also in therapeutic terms, since antagonists are used in the treatment of endocrine disease. Antagonists can block the actions of hormones by simply binding to the receptor without eliciting an effect. In this way, the hormone molecule is prevented from gaining access to the receptor and the response is blocked. The blockage can also occur in the cell, in a number of places. This is illustrated by fig. 3.2. Hormone action in the cell can be blocked by substances such as environmental pollutants which interfere with the processing of the normal intracellular hormone-receptor response. This can occur at a number of sites. The receptor itself can be blocked, or the post-receptor events, such as mRNA or protein synthesis can be inhibited.

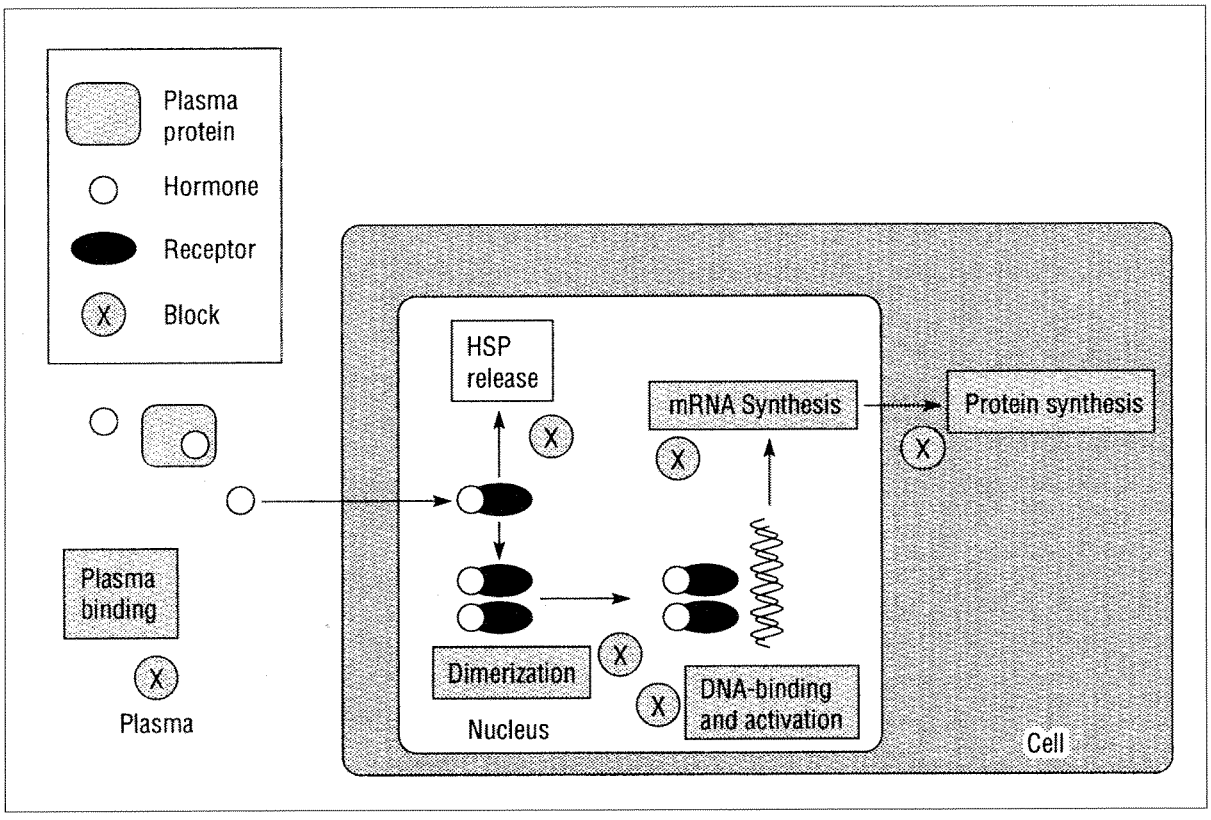

Fig 3.2 Sites of intracellular hormone antagonism

The role of xeno-oestrogens

In the various stages of the endocrine processes, exogenous compounds can disturb the condition of homeostasis. Xeno-oestrogens can enter the organism by various paths, including food-intake via the skin, or via lung or gill.

> Xeno-oestrogens are compounds which can mimic the effect of oestrogen hormone. Strictly spoken, the natural hormones which enter the organisms from the outside, are xeno-oestrogens. In contrast, the endogenous oestrogens are those hormones which have been released by an internal gland of the organism. In this report we maintain this definition, because we consider it the most straightforward approach.

After the uptake, the xeno-oestrogen compounds will be subject to metabolic mechanisms, and only the fraction which is not metabolized will reach the bloodstream.
If xeno-oestrogens reach the bloodstream, they can influence the protein binding process in the bloodstream by competing for the binding sites and thus affect the fraction of hormones which are free to move and activate the oestrogen receptors.

The free fraction of the xeno-oestrogen can easily pass through the biological membranes of the cell and the nucleus because of the lipophilic character of these substances. When the xeno-oestrogen has entered the cell nucleus it can interfere with a number of processes. These processes include the binding between ligand and receptor, the separation of the HSP protein, the dimerization of two receptor proteins and the binding to the DNA. It cannot be excluded that such compounds can even influence the mRNA action and the synthesis of protein. The blockage by the xeno-oestrogen can either be antagonistic or agonistic, i.e., it blocks or potentiates the hormone action through the receptors.

In summary, it is obvious that endocrine disruptors can play a role in the vulnerable balance of the sex hormone system.

Effects on wildlife

Fortunately, organisms have their protection systems in the immune system. Xeno-oestrogens which are bound to the SHBG-proteins are recognized as antigens and an antibody reaction will follow. Larger molecules which move freely in the bloodstream will meet the same fate. In this context it is probable that organisms with a weakened immune system, will be more vulnerable to xeno-oestrogens.

There have been many reports about populations of non-vertebrates, fishes, birds and mammals that suffer from a decline in their reproductive rate. In a large number of cases, this decline is attributed to pollution of the environment by anthropogenic compounds. The xeno-oestrogenic compounds to which the organisms are exposed can influence the morphology, physiology and more specifically the hormone balance of these organisms.

One of the best-known studies is the case of the alligators in Lake Apopca in Florida (USA) (Gui 1994). A chemical spill of organochlorine pesticides was held responsible for the morphologic abnormalities and decreased population of the alligators in comparison to alligator populations in nearby lakes. The oestrogenic hormone levels in females were found to be 50% higher than those in the reference groups.

In the 1970s, the population of the common seal (*Phoca vitulina*) in the Dutch Wadden Sea decreased dramatically compared to populations in the German and Danish parts of the Wadden Sea (Rei 1985). This effect was attributed to the fish diet of the seal in the Dutch waters. The fish contained five times more PCBs than fish caught in the Atlantic Ocean. During the reproductive cycle of the female seals, the increase in the oestrogen concentration was slower than normal at the moment of implantation of the ovum in the womb. Twenty

years later, concentrations of PCBs had decreased by 50 % and the reproductive success of the common seal regained its original level (Rei 1995).

Gulls (*Laridae*) and terns (*Sternidae*) are long-lived, monogamous species, which usually lay 2 or 3 eggs per clutch. The numbers and size of the eggs are reduced when food is scarce. Therefore, when 10% of the nests of western gulls (*Larus occidentalis*) in colonies on the Channel Islands off the coast of southern California were observed to contain 5 or 6 eggs in the late 1960s and early 1970s (Sch 1970), biologists recognized that this was an abnormal phenomenon. Further epidemiological investigations have shown that these subnormal clutches are the product of multiple females engaging in a shared breeding attempt (Hun 1977). These cooperative female-female pairings and polygamous associations are behavioural responses to a female-biased operational sex ratio resulting either from range expansion and colonization or high differential male mortality and/or embryonic feminisation as a result of local environmental factors. The breeding population of western gulls on Santa Barbara Island was severely impacted by organochlorine pollutants, particularly DDT, in the decade prior to the period of highest incidence of female-female pairing, and the breeding population of the colony declined from 3000 birds in 1972 to 850 in 1978 (Sow 1980).

Studies of great blue heron chicks (*Ardea herodias*) from nesting colonies along the Fraser river estuary in British Columbia's Georgia Strait have shown elevated hepatic mixed-function oxidase levels, subcutaneous oedema, growth retardation and morphometric and histological changes in their brains, which correlated with 2,3,7,8-TCDD levels in paired eggs from these nests. At peak contamination, the most contaminated colony failed to produce any young (Ell 1989). Georgia Strait receives wastes from the greatest concentration of forest product industries in Canada; the contamination of fish-eating wildlife in this area is associated with these industries.

The cormorant (*Phalacrocorax carbo sinensis*) which breeds in spring in the Dutch delta area of the Rhine and Meuse basin, has been reported to suffer from high mortality rates among chicks and embryos (Dir 1995). The decline in reproduction which in the late 1970s led to the disappearance of the cormorant populations was attributed to the occurrence of organochlorine compounds in the eggs, such as DDT, its metabolite DDE , PCBs and dioxin-like compounds. It is assumed that the organochlorocarbons influence the birds' hormone and enzyme systems. Now that xeno-oestrogen levels have declined, the cormorant populations have recovered.

Since the beginning of the 1990s, the effluent of sewage treatment works (STWs) in Great Britain caused the induction of plasma vitellogenin in rainbow trout (*Oncorhynchus mykiss*). Vitellogenin is an egg-yolk protein precursor found in oviparous vertebrates, normally only in females. An increased production of this protein in male rainbow trout is indicative of an oestrogenic effect caused by xeno-oestrogens (Job 1993; Har 1999). In addition to increased vitellogenin production male flounder also showed inter-sexuality, which is characterized by the production of egg-cells in normal testes of male specimens (Mat 98).

Whelks, which were abundant twenty-five years ago in the coastal waters of the Netherlands, can nowadays only be found in the Eastern Scheldt. The dog-whelk (*Nucella lapillus*) and the common whelk (*Buccinum undatum*) showed features of imposex. This effect may eventually lead to an overgrowth of the genital papilla by the vas deferens, resulting in sterility and premature death. This phenomenon can be caused by the presence of tributyltin and triphenyltin. The former compound used to be applied as an anti-fouling paint for ships, while the latter is still in use in potato culture. The common whelk is a benthic

predator that lives in the subtidal zone of the seabed. This species has shown an incidence of imposex of over 90% (Men 1996).

Imposex: development of male primary sexual characteristics in female gastropods (also called pseudo-hermaphroditism); female characterics remain intact.

The human-wildlife connection

While there is no doubt about the serious status currently prevalent in our aquatic environment, the risk to human beings from the exposure of xeno-oestrogenic compounds is a topic of debate. The possible link between environmental contamination and human illnesses and disorders is plausible but remains to be proven. However, in order to give the reader an idea of the extent of the possible effects on humans, some comments on the subject are made below.

In considering endocrine disrupting effects, scientists have mainly focused on the consequences for humans in respect of their reproduction. In the beginning of the nineties, a publication by Carlson (Car 1993) made it clear that, based on many studies in the literature, the fertility of the human male had declined by 50 % since World War II. The link with the environment was then quickly made on the basis of what was observed in the aquatic environment (see above). The ensuing fierce debate between supporters and opponents of this theory has continued ever since. Whatever will be the outcome of the debate, the subject is not only relevant to environmentalists. The governmental concern about the presence of endocrine disrupting compounds in the aquatic environment is growing, as is best illustrated by the recently published report by the Dutch Health Council (Gez 1999).

4. Natural and synthetic compounds which show oestrogenic effects

Many organic compounds found in the environment can mimic oestrogenic effects and could in the end have an oestrogenic-disrupting effect. This chapter takes a closer look at the major groups of these compounds and their origins. Based on the chemical and biological data available a risk assessment is carried out on water produced from surface water which may contain these compounds.

There is a group of naturally occurring compounds in the environment, produced by plants and released into the environment. These compounds are called *phyto-oestrogens*.

Another naturally occurring group is that of human and animal hormones. These natural hormones are released into the surface water mainly by water treatment plants or run-off from land in the vicinity of agricultural activity. The sources are pregnant women and farms keeping cows, pigs and chickens. The run-off of the manure spread over the land eventually reaches open water bodies.

Another related group of substances are hormones used as contraceptives. Ethinyloestradiol has been found in the effluent of sewage treatment plants.

By far the largest group of compounds are the industrial or synthetic compounds. In the US, more than 70,000 substances are in daily use; in the EU this number exceeds 100,000. All these compounds have to be screened for their oestrogenic activity to assess the potential threat to humans and the environment. Programmes to investigate this situation are underway.

The main groups of compounds for which it has been established that they have an oestrogen-disrupting effect are:

- Natural and synthetic hormones
- Organochlorine Pesticides (OCP)
- Polychlorinated biphenyls (PCB)
- Dioxins
- Pesticides
- Alkylphenol polyethoxylates (APE) and their metabolites
- Fire retardants
- Phthalates
- Some individual compounds like bisphenol A

4.1 Naturally occurring phyto-oestrogens

An underestimated source of oestrogens are the naturally occurring phyto-oestrogens (Adl 1999). These compounds originate from vegetables and fruits, plants and weeds and belong to the isoflavones, the lignans and other flavones. For instance, white clover (*Trifolium pratense*)and red clover (*Trifolium oepensis)* contain a substance called biochanin A. When fed to sheep in spring, this substance induces a deregulation of the reproduction.

A major example for phyto-oestrogens in food is soy. Soy is consumed by men and domestic animals and contains genistein, an isoflavonoid, which has an oestrogenic effect. Cattle farming in the Netherlands involves an annual consumption of approximately 2 million tons. The beans and soy peelings are given to animals and via the drainage system genistein will eventually reach open waters.

Agricultural activities

In areas with intensive agricultural activities and large concentrations of domestic animals, pollution by natural and synthetic hormones is considerable. The natural hormones are excreted via the urine and faeces of the animals. Compounds of this nature are 17β-oestradiol

and oestron. The synthetic hormones, such as mestranol, are administered to the animals. All these compounds eventually find their way to the surface water via the waste water routing. Another particular source is chicken manure. Dried chicken manure contains oestradiol and is fed to pigs and cows.

4.2 Industrial or synthetic compounds

Organochlorine pesticides have found their way to all corners of the world. Well-known representatives are DDT and its metabolite DDE, lindane and γ-HCH. This group also includes the drins, represented by aldrin and dieldrin. Although these compounds have been banned in the Western world they are still in use in the developing countries, where they constitute a threat to aquatic life. Moreover the depots which have been formed over the years in sediments can be remobilized at any time, depending on local conditions.
Table 4.1 sums up the physical and environmental properties of a selection of these compounds.

Table 4.1 Properties of the major endocrine disruptors showing the distribution coefficient between octanol and water (log K_{ow}), the half-life (DT_{50}) in days, and the bioaccumulation factor in various organisms.

Compound name	log K_{ow}	DT_{50}	Bioaccumulation factor
DDT	-	60	2710 Carp (Cyprinus carpio)
DDE	4.28	60	8450 Carp (Cyprinus carpio)
Aldrin	-	20	12.260 Algae (Chlorella fusca)
Dieldrin	6.2	-	-
PCB	6-7	60-100	10^6 Mussels
Atrazin	2.64	30	3-10 Fish
Nonylphenol ethoxylates	4.2	-	-
Nonylphenol	4.12	6	10 Mussel (Mytilus edulis)
Octylphenol	4.48	-	-
17β-oestradiol	4.01	12	-
17β-ethinyloestradiol	3.67	30	-
Oestron	3.13	-	-
Bisphenol A	3.32	-	-
Dibutylphthalate	4.57	5-15	1500 Glass shrimp(Paleomonetes kadiakensis)
Dimethylphthalate	1.61	3	57 Fish (Lepomis macrochirus)
Diethylphthalate	2.70	3	117 Fish (Lepomis macrochirus)
Di-n-octylphthalate	-	-	9.400 Fish (Gambusia affinis)
Dipropylphthalate	3.27	-	-
Di(2-ethylhexyl)phthalate	9.64	250	130 Fish (Gambusia affinis)

The lipophilicity of these chemicals is generally high. They can pass through membranes without any limit and accumulation in the animal tissues does take place. Consequently the bioaccumulation factors are high and the substances may be a threat for the aquatic life. The products are not easily degraded in the environment and large quantities are still found in sediment. DDT and DDE were the first chemicals which were suspected of xeno-oestrogenic effects.

These considerations are also true for the PCBs. We have seen above that these compounds can easily accumulate in animal tissue and because they are lipophilic ($K_{ow} > 3$) they can pass through biological membranes. The biodegradability in the environment is low; the half-lives of this group of compounds can be as long as 60 to 100 days. Generally speaking these synthetics are highly resistent.

A number of modern pesticides have xeno-oestrogenic properties. This group comprises the chlorotriazines with atrazine as a well-known representative. Another group is the organotinpesticides such as tributyltin and triphenyltin. These agricultural chemicals are more polar: the risk that they bioaccumulate and pass through the membrane of the cell nucleus is smaller. These compounds, however, are very resistant and are not easily degraded. Their lipophilicity is still considerable, and large amounts of these compounds are used.

Relatively new in the field of oestrogenic compounds are the alkylphenol polyethoxylates and their monomers, the alkylphenols. Representatives of these groups include nonylphenolpolyethoxylate, octylphenolpolyethoxylate and the monomers nonylphenol and octylphenol.

The K_{ow} values of these compounds range from 4.2 to 4.5 and they are classified as medium polar. The degradability of alkylphenol ethoxylates is average. Degradation products include the monomers nonylphenol and octylphenol. The products have been found to bioaccumulate in fish.

Phthalates are used as plasticizers in the polymer industry. These substances are very widely used and are found in daily use everywhere. Their properties are less favourable for biomembrane passage. Moreover the compounds degrade relatively swiftly. The huge amounts of these synthetics, however, makes it an important group among the xeno-oestrogens.

There is yet another single compound which is important in this context: bisphenol A. This chemical is in use as an additive in the polycarboxylate industry, for the production of plastic bottles.

Risk assessment of chemical compounds

The properties listed in table 4.1 allow the potential penetration risk of the substances to be derived. Substances which have a high log K_{ow} value and which are persistent in the environment have a large bioaccumulation factor. The table shows that DDT and DDE show this effect in fish (carp) with bioaccumulation factors of 2710 and 8450 respectively. Such substances are a potential risk.

The table below shows the relative potential of xeno-oestrogens in the ERC test. The last column indicates the Non Observed Effect Concentration (NOEC) for the substances. These NOEC data have been derived from the literature and indicate only effects related to the endocrine system or to physiological changes in the reproductive system.

The ratio between the predicted environmental concentration (PEC) and the NOEC is generally used as a measure for the potential risk of a substance. If this ratio is greater than 1, the substance is considered a risk substance. In their July 1999 report (Gez 1999) the Health Council of the Netherlands used an arbitrary safety factor of 100 in order to account for synergistic effects between substances. The present report also uses this factor. For the PEC, we only use measured concentrations.

The NOEC data and the actually determined concentrations allow the total PEC/NOEC of each substance or group of substances to be estimated. This ratio, in combination with the estimated bioavailability and the actual concentration multiplied by the half-life in days and the Log K_{ow}, offers a useful set of figures which leads to a meaningful interpretation in the next chapters.

Table 4.2 Relative oestrogenic potential of a number of endocrine disruptors in the ERC assay, expressed as ng/l - 17β-oestradiol equivalents (Leg 1999), and the calculated No Observed Effect Concentration (NOEC) in µg/l. EEF = 17β-oestradiol equivalent factor.

Compound name	EEF ERC	NOEC
17β-oestradiol	1	3×10^{-4} (ref. Leg 1999)
oestron	0.058	5.2×10^{-3}
17α-oestradiol	0.016	1.9×10^{-3}
17α-ethinyloestradiol	0.81	4×10^{-4}
bisphenol A	7.8×10^{-6}	38.5
dimethylphthalate	1.1×10^{-5}	27.3
diethylphthalate	3.2×10^{-8}	9.3×10^{3}
di-n-butylphthalate	1.8×10^{-8}	$1,7 \times 10^{4}$
butylbenzylphthalate	1.4×10^{-6}	213
dioctylphthalate	$< 3 \times 10^{-8}$	1×10^{4}
4-nonylphenol	3.8×10^{-5}	8.8
4-octylphenol	1.4×10^{-6}	213
o,p DDT	9.1×10^{-6}	33.0
dieldrin	2.4×10^{-7}	1250
genistein	6.0×10^{-6}	50

The individual response of a substance in the ERC assay can be related to the actual concentration. The product of the concentration and the EEF from table 4.2 yields the oestrogenic effect per substance, expressed as ng/l - 17β-oestradiol equivalents (EEQ). The calculated response was compared with the actual response of the ERC bio assay, which was carried out in the water samples. This allowed an estimate to be given for each individual compound of the total oestrogenic potential of a sample (see chapter 7). This method disregards synergistic or antagonistic effects.

The response of the ERC assay is higher than that of *in vivo* tests such as the vitellogenin test carried out by Routledge (Rou 1998). This is due to the fact that the substances do not need to pass a cell wall barrier. When samples are measured anti-oestrogenics contribute also to the response of the test. The NOEC value for 17β-oestradiol is the response determined with the ERC assay and equals 0.3 ng/l. The NOEC values of the other compounds are calculated by dividing this value by the EEF's of the compounds.

5. Current status regarding the presence of xeno-oestrogenic compounds in surface and potable water and potential risk for purification processes

Xeno-oestrogenic compounds have been found in surface and drinking waters in measurable quantities over the last decades (Den 1998). Depending on their solubility in water, these chemicals are transported by the water and distributed over large distances. The object of the present study - as is explained in Chapter 6 – is to restrict the analysis to those products which have a proven oestrogenic effect and which can still be expected to occur in the environment. This chapter comprises those substances or groups of substances which have been actively measured in the RIWA subproject.

Hormones

In the past, hormones and the synthetic hormones have been found in drinking water. In 1977, Rurainski (Rur 1977) and co-workers measured 17α-ethinyloestradiol and 17β-oestradiol in drinking water in south-western of Germany. The concentration range was 0-22 ng/l for ethinyloestradiol and 0-0.94 ng/l for 17β-oestradiol. More recent reports do not confirm this observation (Stu 1996) and only demonstrate the presence of hormones in surface water.

Table 5.1 summarizes the concentrations of 17β-oestradiol, oestron and 17α-ethinyloestradiol.

Recently, Belfroid (Bel 1999) has shown the presence of hormones in the rivers Meuse and Rhine in the Netherlands: a maximum concentration of 2.8 ng/l was found for 17β oestradiol and 4.3 ng/l for oestrone. When samples were taken in the vicinity of the outlets of waste water treatment plants, the maximum concentration for 17β-oestradiol could increase to 12 ng/l.

Table 5.1 Reported concentrations of natural and synthetic hormones in surface waters in the Meuse and Rhine basin. Data shown are maximum concentrations in ng/l.

	17β-oestradiol	**Oestrone**	**17α-ethinyloestradiol**
Rhine and tributaries Germany (Stu 1999)	-	-	4
Rhine (Dutch part) (Bel 1999)	5.5	3.4	-
Meuse (Dutch part) (Bel 1999)	2.8	4.3	2.9
Rhine and side rivers Germany (Ter 1999)	-	1.6	-
Various rivers in Germany (Weg 1999)		-	4 -

Concentrations in the Rhine and its tributaries in Germany show similar values: oestron concentrations ranged from 1 to 4 ng/l. The hormone 17β-oestradiol has not been found in surface water of the Rhine. The synthetic hormone 17α-ethinyloestradiol has occasionally been detected (Stu 1996) or not at all (Ter 1999).

Bisphenol A
Bisphenol A is used in the production of plastics and epoxy resins. In the winter of 1997, the concentration at Eijsden (Meuse) reached levels of 150 ng/l and that at Lobith (Rhine) 25 ng/l. The concentration in the effluent of STPs is between 20 and 100 ng/l (Bel 2000).

Phthalates
Phthalates are used in huge quantities as plasticizers and for that reason they are found everywhere in the environment. In a 1997 pilot study of the LOES project, a number of phthalates were observed. Concentrations of phthalates in Dutch surface water have been found to be in the range of 0.1 to 3.5 µg/l. This highest phthalate concentration in the Netherlands was that of di(2-ethylhexyl)phthalate, which is the most commonly found phthalate (Bel 2000).

Alkylphenol polyethoxylates (APE) and their metabolites
The alkylphenol polyethoxylates (APEs) are a large group of nonionic surfactants used for the manufacture of detergents and detergent-based products, and have been discovered to degrade into various smaller alkylphenolic chemicals which can act as oestrogen mimics. Of these products, over 300,000 tons a year are used worldwide. The APEs and their degradation products have been found in both the Rhine and Meuse in the vicinity of industrial sewage discharge into the rivers. Values for nonylphenolethoxylates ranged between 0.9 and 15 µg/l. In surface waters, however, the nonylphenol concentration did not exceed 0.14 µg/l (Bel 2000).

In the summer and autumn of 1984 and 1985 concentrations of APE were measured in the Chriesbach, a small creek discharging in the Glatt River which is a tributary of the Rhine. The average water concentration of nonylphenol (NP) was 3.9 µg/l, that of nonylphenolmonoethoxylate 24 µg/l and that of nonylphenol diethoxylate 9.4 µg/l (Ahe 1993).

6. Experimental part of the project

The goal of the RIWA sub-project was to determine the concentration of a selected number of natural and synthetic endocrine disrupting compounds in various water bodies and to determine the oestrogenic effect of the samples by bio assay methods.

An additional goal of this study was to determine the correlation between the analytical results and the results of the bio assays.

The samples were taken in three separate rounds: in March, June and September. The samples were collected at the sampling locations which are also used in RIWA's routine monitoring programmes. Samples were transported to the various laboratories for sample preparation and analysis. The data were centrally collected by RIZA in a results database. The present report is based on the data owned by RIWA, supplemented with data from the RIZA sample sites Lobith and Eijsden.

6.1 Choice of sampling locations

The part of the project assigned to RIWA, entitled 'Study on the occurrence of endocrine disrupting compounds in surface, process and drinking water in the Rhine and Meuse basins', comprises a number of criteria for the choice of the sampling locations (see fig. 6.1).

The sampling locations were chosen on the basis of RIWA's existing monitoring network. For many years now, RIWA and the participating water companies have been monitoring the quality of the Rhine and Meuse surface water for a large number of chemical and biological parameters. The frequency of this monitoring programme varies, but is roughly monthly. The RIWA network includes two sampling locations owned by RIZA on the Dutch border. The present study involved these two locations at Lobith – at the Dutch-German border on the Rhine embankment- and at Eysden on the Dutch-Belgian border on the river Meuse.

The criteria for the surface water locations were:

- The sampling locations had to be situated in the Rhine and Meuse basins, in Germany, Belgium or the Netherlands;
- Samples had to be collected from these locations at least every month, to allow monitoring programmes on a broad package of parameters;
- It had to be possible to change the selection of locations depending on the results of the first and/or the second phase of the project.

The criteria for the samples taken from the treatment facilities of the water companies in the various stages of the purification process were:

- The sampling locations had to be situated at the drinking water production facilities of the participating Dutch RIWA companies.
- Samples had to be collected from these locations frequently, to allow monitoring programmes on a broad package of parameters.
- The drinking water samples (tap water quality) had to be withdrawn at sampling points just behind the pumping-stations.
- It had to be possible to change the selection of locations depending on the results of the first and/or the second phase of the project.

Table 6.1 Summary of the sampling locations used by RIWA in the LOES project

Location	Company name	Description	Phase 1	2	3	Sample code
Meuse, *Surface water*						
Remilly	BIWM	French-Belgian border	X	X	X	W.OW.n.REM.xx.F
Tailfer	BIWM	Meuse: BIWM inlet	X	X	X	W.OW.n.TAI.xxx.F
Namêche	AWW	Meuse	X	X	X	W.OW.n.NAM.xxx.F
Liège	AWW	Albert kanaal	X	X	X	W.OW.n.LUI.xxx.F
Eijsden	RIZA	Belgian-Dutch border	X	X	X	A.OW.n.EYS.xxx.F
Heel	WML	Lateraalkanaal at Heel production facility		X	X	W.OW.n.WPH.xxx.F
Roosteren	WML	Grensmaas at Roosteren production facility		X	X	W.OW.n.WPR.xxx.F
Belfeld	RW-DL	Meuse (NL)	X	X	X	W.OW.n.BEL.xxx.F
Keizersveer	RW-DZH	Meuse: WBB and DZH reference sites	X	X	X	W.OW.n.KEI.xxx.F
Biesbosch	WBB	Gat van de Kerksloot: WBB inlet	X	X	X	W.OW.n.WBB.xxx.F
Brakel	DZH	Inlet at Afgedamde Maas	X	X	X	W.OW.n.BRA.xxx.F
Processed water						
Petrusplaat	WBB	Finished product	X	X	X	W.LW.n.WBB.xxx.O
Scheveningen	DZH	Collected after filtration; drinking water	X	X	X	W.LW.n.DZ1.xxx.O
Scheveningen	DZH	Collected after extraction	X	X	X	W.LW.n.DZ2.xxx.O
Ouddorp	Delta	Collected process water		X	X	W.OW.n.OUD.xxx.F
Ouddorp	Delta	Drinking water		X	X	W.LW.n.OUD.xxx.O
Braakman	Delta	Drinking water		X	X	W.LW.n.BRA.xxx.O
Kralingen	WBE	Drinking water		X	X	W.LW.n.WB1.xxx.O
Berenplaat	WBE	Drinking water		X	X	W.LW.n.WB2.xxx.O
Rhine, *Surface water*						
Karlsruhe	TZW	Rhine (D)	X			W.OW.n.KRL.xxx.F
Köln	GEW	Site 1	X			W.OW.n.KO1.xxx.F
Köln	GEW	Site 2	X			W.OW.n.KO2.xxx.F
Köln	GEW	Site 3	X			W.OW.n.KO3.xxx.F
Köln	GEW	Site 4	X			W.OW.n.KO4.xxx.F
Köln	GEW	Site 5	X			W.OW.n.KO5.xxx.F
Köln	GEW	Site 6	X			W.OW.n.KO6.xxx.F
Lobith	RIZA	German-Dutch border	X	X	X	W.OW.n.LOB.xxx.F
Nieuwegein	WRK	Lekkanaal: inlet	X	X	X	W.OW.n.LKN.xxx.F
A'dam Rijn kan.	GWA	Weesperkarspel inlet			X	W.OW.n.GW1.xxx.F
Andijk	PWN	Inlet at IJsselmeer	X	X	X	W.OW.n.AND.xxx.F
Twentekanaal	WMO	WMO inlet	X	X	X	W.OW.n.WMO.xxx.F
Processed water						
Nieuwegein	WRK	Outgoing water	X	X	X	W.LW.n.LKN.xxx.O
Lekkerkerk	WZHO	GLS PF 99A (Lekkerkerk)	X	X	X	W.OW.n.WZ1.xxx.F
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	X	X	X	W.OW.n.WZ2.xxx.F
Leiduin	GWA	Drinking water	X	X	X	W.LW.n.GW1.xxx.O
Leiduin	GWA	Recharged after infiltration (a.o.)	X	X	X	W.LW.n.GW2.xxx.O
Leiduin	GWA	After ozone treatment (a.o.)	X	X		W.LW.n.GW3.xxx.O
Leiduin	GWA	After active carbon filtration (a.c.)	X			W.LW.n.GW4.xxx.O
Weesperkarspel	GWA	Drinking water		X		W.LW.n.GW4.xxx.O
Weesperkarspel	GWA	Drinking water			X	W.LW.n.GW3.xxx.O
Andijk	PWN	Drinking water	X	X	X	W.LW.n.AND.xxx.O
Twentekanaal	WMO	Drinking water	X	X	X	W.LW.n.WMO.xxx.O

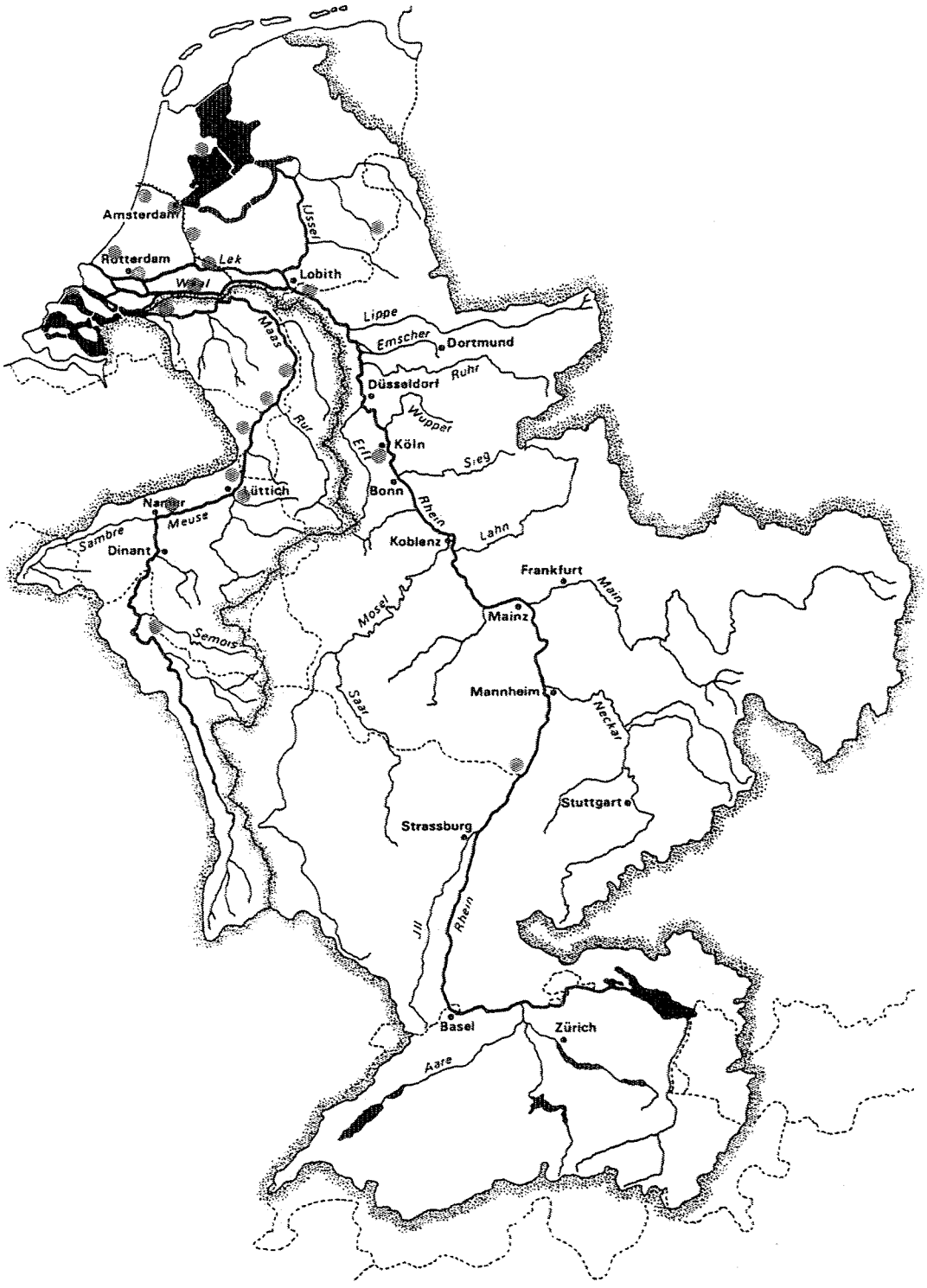

Fig. 6.1 Map of the Meuse and Rhine basin with sampling locations indicated by red dots.

The LOES project aimed at taking samples in three seasons in 1999: late winter (beginning of March), early summer (end of June) and autumn (September). The sampling campaign was divided into three sampling tours or phases, indicated as phase 1, phase 2 and phase 3.

The coordination of the sampling was in the hands of RIZA and the transportation was carried out by TAUW in Deventer, The Netherlands.

The majority of sampling sites were visited three times. However, as was mentioned above, one of RIWA's goals was to allow the participating water companies to request new sampling points or to alter the locations during the project. The number of sampling points could therefore vary with the phase.

Table 6.1 summarizes the sampling locations, providing the name of each location together with a short description of the site and the corresponding sample code.

Abbreviations used:

BIWM	Brusselse Intercommunale Watermaatschappij
Delta	NV Delta Nutsbedrijven
DZH	NV Duinwaterbedrijf Zuid-Holland
GEW	Gas Elektricität und Wasser-Werke Köln AG
GWA	Gemeentewaterleidingen Amsterdam
AWW	Intercommunale Vennootschap Antwerpse Waterwerken
PWN	NV PWN Waterleidingbedrijf Noord-Holland
RIWA	Samenwerkende Rijn- en Maaswaterleidingbedrijven
RIZA	Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling.
RW-DL	Rijkswaterstaat – Directie Limburg
RW-DZH	Rijkswaterstaat – Directie Zuid Holland
WBB	NV Waterwinningbedrijf Brabantse Biesbosch
WBE	NV Waterbedrijf Europoort
WMO	Waterleiding Maatschappij Overijssel NV
WRK	NV Watertransportmaatschappij Rijn-Kennemerland
WZHO	NV Watermaatschappij Zuid-Holland Oost

The sample code protocol

The sample code contains several items of information. The general form of the code is:

X.YY.n.ZZZ.xxx.y: an example is W.OW.2.KEI.APE.F

1. The first position (X) is allocated to the client: W for RIWA and A for RIZA.
2. The next two positions (YY) indicate the sample compartment: OW for surface water; LW for the other types of water.
3. The next position (n) indicates the phase number: 1,2 or 3 (in actual samples).
4. Three positions (ZZZ) indicate the sampling location (see table 6.1).
5. The three subsequent positions (xxx) indicate the type of analysis:
   ALW = general parameters: chlorine, salinity
   APE = Alkylphenol polyethoxylates
   ERC = Estrogen CALUX test
   FTA = Phthalates
   HOR = Hormones and Bisphenol A
   YES = YES + ERC (YES has not been performed; some samples taken for YES have been used for ERC)
   ZS = particulate matter, dry residue

6. The last single position shows the sample treatment procedure: O means no treatment, F means filtration.

6.1.1 Sampling and sample treatment

For the sampling of surface water a stainless steel bucket was used and samples were poured into glass bottles by a stainless steel funnel (RWS protocol 913.00.W001). Subsequently the water samples were filtered. The filtration procedures applied depended on the analytical method involved. The description can be found in the sections explaining the analytical methods.

Process and drinking water was directly tapped from sampling taps. Samples were collected in green glass bottles of 250 to 1000 ml. The bottles were used with caps which had Teflon inlays. The samples were not filtered before analysis.

During sampling and handling use of plastic tubing, gloves and other equipment was avoided.

6.2 Selection of measuring methods

The compounds selected for the present study are those considered all over the world as causing endocrine problems. The analytical methods applied in the project were primarily chosen on the basis of the results of a pilot project which was carried out in 1998. This pilot project treated the reliability of the analytical methods.

Substances which are regularly measured in RIWA's operational measuring programmes, such as organochlorine pesticides, were not taken into account, since these substances are hardly ever found in the Rhine and Meuse basins. The set of compounds chosen by RIWA were: hormones, alkylpolyethoxylates and alkylphenols, and phthalates. The ERC assay was chosen as the bioanalytical sum parameter. Fireretardants were not included because their contribution to the endocrine effect in the water compartment was expected to be low.

The sample preparation for the ERC assay was tested separately in the pilot study: the water samples were to be prepared in such a way that the ERC assay could be interpreted satisfactorily.

6.3 Description of the measuring methods

This paragraph describes the analytical methods which were applied in the RIWA project. In the case of the ERC assay, an extensive study was carried out in 1998 in order to establish the most appropriate sample preparation technique. It was found that the sample preparation performed by the MTC lab in Amsterdam gave the best results. In the present project, the ERC assay samples were first transferred to the MTC lab for sample preparation and the resulting extracts were transported to the LUW lab. Since the YES (Yeast Estrogen Screen) test, which originally plannend, was not performed, some samples taken for YES were used for the ERC assay.

6.3.1 The ER-CALUX bio assay (ERC bio assay)

Carried out by the Department of Toxicology, Agricultural University Wageningen, The Netherlands under the responsibility of A.J. Murk

Description of the method

De ER-CALUX ('estrogenic receptor chemically activated luciferase gene expression') assay is an *in vitro* bio assay which enables screening of compounds and extracts on their

oestrogenic potential. If an extract contains a mixture of oestrogenic compounds, the combined effect will be strengthened. Unlike chemical methods, this assay enables combined biological effects to be measured.

The ERC assay has recently been developed in the Netherlands (Leg 1999) and is very sensitive and able to measure anti-oestrogenic effects. The assay provides a measure in the total oestrogenic effect.

The first step is to bind the substance to the oestrogenic receptor in the cytoplasm of the cell. After this binding, the oestrogenic receptor is activated, after which a complex of two receptors is formed (dimerization) and the translocation of the complex to the cell nucleus is accomplished. In the nucleus the dimer binds to a specific part of DNA, the ERE (oestrogenic receptor element) and the transcription of the gene proceeds. This train of events can be influenced by a single substance which reaches the cell and consequently alters the function of the cell and the physiology of the organism.

Principle of the method

The ERC assay is carried out with a recombinant humane T47D breast adenocarcinoma cell line which contains original ER and includes a stable ER-mediated firefly (*Photinus pyralis*) luciferase gene expression.

When pseudo-oestrogenic compounds are brought into contact with the cell, they pass through the cell wall and bind to the human oestrogenic receptor and activate the receptor; the dimer then binds to the ERE. Consequently, luciferase will be formed in the cell and its quantity is a direct measure of the oestrogenic potential of the substance or extract being studied. The addition of the luciferine substrate yields a quantity of light which is proportional to the quantity of luciferase. This light is measured by a luminometer.

Experimental method (Mur 1999)

The cells were seeded on a standard microtiter plate in 100 µl assay medium. Unlike the normal growth medium (culture medium), this medium contains no hormones, particularly no oestradiol, because they would disturb the method. In order to remove the hormones, the cell plate was refreshed with assay medium after 24 hours and again shortly before starting the test with the sample.

After this preparation procedure the cell medium was replaced by the sample extract and the cells were exposed for 24 hours. Subsequently, the cells were washed with diluted buffer and a lysis buffer was added with a very low osmolarity in order to promote swelling. The swollen cells were frozen at a temperature of minus 80 degrees Celsius. These conditions ruptured the cell walls and the luciferase formed was released form the cell into the medium.

Luminescence measurement

The luciferine substrate was automatically added and the light was measured in a luminometer. The quantity of light measured was interpolated in the calibration curve for oestradiol and was reported in oestradiol equivalents (EEQ).

6.3.2 Method for the analysis of hormones and bisphenol A

Carried out by the Institute of Environmental Studies of the Free University in Amsterdam under the responsibility of A.C. Belfroid.

Principle of the method

The analysis of oestrogenic hormones and bisphenol A was carried out as described by Belfroid et al (Bel 1999), with the exception that the quantification of the chromatograms was carried out with the internal deuterated standard of d^4-17β oestradiol and d^6-bisphenol A.

According to the analytical procedure all samples were filtered prior to extraction. However, the drinking water samples not filtered and the whole sample was used for the extraction. Samples of 1 litre were extracted by solid phase extraction, and further clean-up was performed by liquid chromatography. The fractions containing the analytes were silylated and the components were determined by GC-MS/MS. This method allows the components 17β-oestradiol, 17α-oestradiol, oestron, 17β-ethinyloestradiol and bisphenol A to be determined.

Experimental method (Bel 1999, modified with a number of amendments by the author)
Sample pretreatment
Prior to filtration, the samples were spiked with the deuterated internal standards d^4-17β oestradiol and d^6-bisphenol A. Samples consisting of 1 l surface water or waste water were filtered over combined 0.45-μm and 1.2-μm filters. The compounds were extracted with an acetone and methanol activated SDB-XC disk in a Varian disk extraction apparatus. The compounds were eluted from the disk with 3 x 5 ml methanol. The methanol was subsequently evaporated at 60 °C under nitrogen and the residue (max. 100 μl) was purified over a combination of C_{18} and NH_2 columns (Baker), which had been activated with methanol/water and ethylacetate, respectively. The hormones were eluted with 3 x 1 ml ethylacetate. The ethylacetate was evaporated under nitrogen to dryness at 60 ° C. The residue was dissolved in HPLC eluent (methanol/water 65/35) and fractionated with HPLC with a 15 cm x 4.6 mm I.D.S5 PAH column (manufactured by Phase Separations) with methanol/water 65/35 as the mobile phase flowing at 1 ml/min. The hormones were eluted after ~8-11 min and Bisphenol A after 3-4 min. Fractions containing the hormones were collected, evaporated until dryness at 60°C and silyated with SIL A reagent for 1 h at 60 °C. After evaporation, the residue was dissolved in hexane together with the injection standard PCB103. This mixture was then washed with water in order to remove harmful by-products that would contaminate the GC column or detector. The hexane phase was dried over a sodium sulphate column and collected in a GC vial. The hexane fraction was evaporated to 50 μl, of which 3 μl were used for GC-MS/MS.

GC-MS/MS analysis
The complex formed was measured with GC-MS/MS (Varian 3400 GC with a Saturn IV ion-trap mass spectrometer), equipped with a 30-m DB-5MS column (0.25 mm I.D., 0.25 μm film). Since the silylation reaction resulted in aggressive by-products, the GC column was protected with a 2 m retention gap of deactivated fused silica 0.53 I.D.
The limit of detection (LOD) for the oestrogenic hormones was set at three times the noise level of the baseline in the chromatogram and is established per series of analysis. For Bisphenol A the the LOD was based on three times the standard deviation of the long term average of the bisphenol A level in control samples. The limit of quantification (LOQ) was set at three times the LOD. Values between LOD and LOQ are indicated in the tables by '<*'. The LOD varies with the series, which means there are different LOD values for different series.
The detection limit for all RIWA samples were 0.3 ng/l for oestron, 17α-oestradiol and 17α-ethinyloestradiol, 0.8 ng/l for 17β-oestradiol and 12 ng/l for Bisphenol A.

6.3.3 Method for the analysis of phthalates in environmental samples (Vel 2000)

Carried out by the Laboratory for Organic-Analytical Chemistry of the National Institute of Public Health and the Environment in Bilthoven, The Netherlands, under the responsibility of E. van der Velde

Principle of the method
The water samples were filtered over glass fibre filters to obtain the water soluble fraction and only some particle bound fraction on small particles (<1.2 μm). Nine single isomer phthalates

were determined in water samples using solid phase extraction (SPE) and analysis involving gas chromatography with mass selective detection (GC-MS). Quantification was performed using deuterated internal standards for the phthalates and an injection standard. In analyzing phthalates, attention was paid during the whole procedure and in all steps to prevent contamination with phthalates from glassware, laboratory equipment, solvents etc.

Experimental method

Reagents

The following single isomer phthalates were analyzed: dimethylphthalate (DMP*), diethylphthalate (DEP*), dipropylphthalate (DPP), dimethylpropylphthalate (DMPP), dibutylphthalate (DBP*), butylbenzylphthalate (BBP*), dicyclohexylphthalate (DCHP), diethylhexylphthalate (DEHP*), di-n-octylphthalate (DOP*). For the phthalates indicated by an '*', a deuterated phthalate was used for quantification and correction of recovery.
Diallylphthalate (DAP) was used as the injection standard.
All solvents had to be of high quality and were checked before use for any contamination with phthalates. All glassware was cleaned with dichloromethane before use. The use of plastic materials and gloves during sample pretreatment was avoided.

Sample pretreatment

The samples were taken in new 1 l bottles with a screw cap and PTFE inlay. Samples were stored at 4 °C and were analyzed within four days.
Surface water samples were homogenized and 250 ml of the water were filtered over a precleaned 1.2 µm glass fibre filter (GF/C); drinking water samples were not filtered. 10 µl of the internal standard solution of deuterated phthalates (ca 100 µg/ml) were added and the sample was introduced in a SPE column filled with 500 mg RP-C18PolarPlus material using full-glass equipment. The SPE column was eluted with 8 ml of pentane/MTBE (70/30). The extract was concentrated to 1 ml and 10 µl of the internal standard solution of diallylphthalate (ca. 100 µg/ml) were added.
In each series of samples, two procedural blanks (containing only internal standard solution, because it was not possible to find phthalate-free water) were analyzed using the full procedure, together with the samples used to check for contamination with phthalates.

GC-MS analysis

1-2 µl of extract was injected on-column on a CPSil5CB (25m * 0.25 mm ID; 0.25 µm film) column on a gas chromatograph (Fisons 8000) using mass selective detection (MD 800). Helium was used as the carrier gas with a constant gas flow of 1 ml/min.; the injection temperature was 60 °C. The temperature programme started at 60 °C(0.5 min) and raised the temperature by 20 °C/min to 100 °C and then by 10 °C/min to the final temperature of 270 °C. Ionisation was performed under electron impact (EI) conditions using 70 eV. The interface temperature was 270 °C and the EI source was 250 °C. The components were analyzed in SIR mode at m/z 163 for DMP and m/z 149 for the other phthalates (m/z 167 for D4-DMP and m/z 153 for the other deuterated phthalates).
Identification and quantification was performed on the basis of retention times, mass selective detection and peak area, assuming that the response of the deuterated phthalates corresponded with that of the normal phthalates. Quantification on the deuterated phthalates meant that the values were directly corrected for recovery and afterwards for the mean value of the procedural blanks analyzed in the same series. For the other phthalates, quantification was performed with external standards.

Quality control

The linearity of the instrument was established over the range from 0.1-25 µg/ml. Procedural blanks were injected three times during each series of samples and instrumental blanks

(solvents) were analyzed as well to check the contamination of the instrument. The procedural blanks were between 0.01 and 0.06 µg/l. The limit of determination (LOD) was calculated from the standard deviation of the procedural blanks and might vary slightly between the series of samples. In general, the LOD for water samples was between 0.01 and 0.18 µg/l.
The repeatability of the procedure is between 5 and 10 %. The recovery rates were between 50 and 102 %, depending on the types of water analyzed.

6.3.4 Method for the analysis of alkyphenol ethoxylates in water samples (Voo 2000)

Carried out by the MTC lab in Amsterdam under the responsibility of P. de Voogd

Principle of the method

The water samples were filtered and extracted over a solid phase extraction cartridge. After clean-up over an adsorption-chromatographic column, the samples were quantified on a RP-HPLC system, after matching with the appropriate reference sample on a NP-HPLC system. This method allowed nonylphenol ethoxylates, octylphenol ethoxylates, nonylphenol and octylphenol to be analyzed.

Experimental method

Sample pretreatment

After delivery, the water samples were registered and stored at 4 °C in a cooling chamber.
Within 20 days, the surface water samples were filtered and extracted. The other water samples were extracted without filtering. Filtration took place over Whatman GF/C glass fibre filters (1.2 µm) and the filtrate was collected in an Erlenmeyer flask. The volume prepared was amply sufficient for further preparation.
Before extraction the water samples were brought to room temperature, and a mass of between 60 and 600 grams accurately weighed; subsequently a volume of at least 35 ml of methanol was added.
The extraction was carried out with SPE-C18 cartridges provided with glass wool and coupled to a water reservoir. The samples were poured on the column and elution took place under pressure and if necessary in portions. The water eluate was discarded. The SPE column was eluted with methanol and the eluate was collected in a sample vial. The sample was put in a water bath and the solvent evaporated to dryness. The residue was dissolved in a mixture of dichloromethane: hexane of 1:3 v:v.

Sample purification

The extract was then purified by adsorption chromatography over a combined column consisting of: (1) Aluminum oxide (neutral, 0.063-0.200 mm, 70-230 mesh ASTM), deactivated with 5% w/w water; (2) Silica (230-400 mesh ASTM), impregnated with $AgNO_3$ and (3) sodium sulphate dehydrate. The column was eluted with a dichloromethane:hexane of 1:3 v:v; the eluate was discarded. The next fraction was eluted with a dichloromethane:hexane mixture of 8:2 v:v. This fraction was collected, since it contained the analytes of interest, viz. the alkylphenol ethoxylates or the alkyl phenols. After evaporation of the solvent the extract was ready for HPLC analysis.

HPLC analysis

Quantification of the APE was based on the assumption that the mean length of the ethoxylate chain in the APE mixture was equal to that of the standard mixture used. If this is the case, then the sum of the individual ethoxylates which can be quantified with the NP-HPLC system is approximately equal to the quantification obtained with the RP-HPLC system. In this system the eluting peaks were integrated. For verification, every sample was analyzed by NP-HPLC (qualitatively) and by RP-HPLC. The quantification of the integrated sample peak was carried out

by comparing the sample with a standard mixture. The mixture showing the best match with the sample in NP-HPLC was used for quantification in RP-HPLC.
The HPLC system consisted of a Waters 600^{E} System Controller, a Waters 717plus Autosampler and a Waters 474 scanning fluorescence detector.
Reversed phase Conditions: Column: Lichrospher 100 RP-18, 125 x 4 mm (Phenomenex, D). Mobile phase: MeOH: water 80: 20 v/v, isocratic elution, flow rate of 1.0 $ml.min^{-1}$. Detection: fluorescence, excitation 230 nm, emission 290 nm.
Normal phase Conditions: A Hypersil NH_3 (3 μm) 100 x 4.6 mm (Phenomenex, D) column. Mobile phase composition: Gradient elution of 98:2:0 tot 0:97:3 hexane:propanol:water at a flow rate of 1.5 ml. min^{-1}. Detection as RP-HPLC.

6.4 Treatment of the resulting data

The results of these analyses are listed in the tables in Appendix 1. The tables are arranged per phase i.e. March, June and September, and subsequently per parameter: ERC assay, hormones and bisphenol A, phthalates and alkylphenol polyethoxylates.

The tables are further divided into the Meuse and Rhine samples, while the surface water samples are listed from upstream to downstream. To preserve a clear overview, the process water samples are grouped together, as are the drinking water samples.

In the figures constructed from the results, the non-detects have been treated in the usual manner: results below the detection limit were entered as the detection limit divided by two; results equal to zero and results lower than zero were considered to be zero.

7. Interpretation of the results and general discussion

This chapter discusses the results of the individual parameters and the meaning for the safe production of drinking water from surface water. In order to establish this it is studied whether xeno-oestrogen are present and if there is a oestrogenic potential. Furthermore we will be looking into the relation between oestrogenic potential of water samples and the concentrations of individual substances which could be responsible for this effect. If relevant we will be looking at the possibility of the occurrence of 'hot spots'. This knowledge will be used to assess the risks of producing drinking water from surface water. The potential threat at the water intake is discussed in relation to the effectiveness of the individual purification processes of the water companies.

Collection of the water samples started in the early spring of 1999. At various locations along the Meuse and Rhine rivers, samples were taken from the surface water and from various stages in the drinking water production process of the water supply companies that use river water as their source. This operation was repeated twice: in June 1999 and in September 1999. The sampling dates were the same at every sampling point, with a few exceptions. This means that the analysis data give information about a momentary situation.

The sampling sites in each period or phase may differ from each other, since the participating waterworks were allowed to change their sampling points between phases. As a consequence there is only limited information available for those sampling points and conclusions can hardly be made.

The samples were transported to various laboratories and analyzed on individual xeno-oestrogens and oestrogenic potential. The final results were available in the beginning of 2000. The results of these analyses are listed in the tables in appendix 1. The tables are arranged per phase, i.e. March, June and September, and subsequently per parameter: ERC assay, hormones and bisphenol A, phthalates and alkylphenol polyethoxylates and general parameters. The tables are further divided into the Meuse and Rhine samples, while the surface water samples are listed from upstream to downstream. To preserve a clear overview, the process water samples are grouped together, as are the drinking water samples.

7.1.1 Comparison between Meuse and Rhine

For the discussion in this chapter, the median concentration data from the tables in Appendix 1 have been summarized in table 7.1. The table shows the median of concentrations for a number of parameters in Meuse and Rhine at the time of sample collection in March, June and September (Phase 1, 2 and 3).

Differences were tested using the Mann-Whitney-U test, significance level 5%; significant differences are indicated by an asterix, non-significant comparisons with a dash). P1, P2 and P3 indicate the three sample phases of the project in March, June and September 1999. Table 7.1 summarizes the results of this test, which are discussed below in the context of the various products.

At first glance, the median concentrations in the Meuse appear to be higher than those in the Rhine. The Mann-Whitney-U test shows that the median phthalate concentration was significantly higher in the Meuse, but the bisphenol A concentration was not. The oestrogenic potential expresssed by the ERC test, is significantly higher in the Meuse than in the Rhine.

Table 7.1. Comparison between several water types of the rivers Meuse and Rhine and their products. (proc. dr. water = process and drinking water, prepared from surface water extracted from the rivers Meuse or Rhine.) Median concentrations phthalates in µg/l, bisphenol A in ng/l, ERC in ng/l EEQ. Differences were tested using the Mann-Whitney U test, significance level 5%; significant differences are indicated by an asterix, non-significant comparisons with a dash). P1, P2 and P3 indicate the three sample phases of the project in March, June and September 1999.

Component	type of water	median	type of water	median	significance
ERC	Meuse (P1-P2)	0.0132	Rhine (P1-P2)	0.0054	*
Bisphenol A	Meuse (P1-P3)	13.5	Rhine (P1-P3)	17.5	-
Bisphenol A	Meuse (P1-P3)	13.5	Meuse proc. dr.water (P1-P3)	5.5	*
Bisphenol A	Rhine (P1-P3)	17.5	Rhine proc. dr.water (P1-P3)	5.45	*
Phthalate	Meuse (P1-P3)	1.61	Rhine (P1-P3)	0,99	*
Phthalate	Meuse (P2)	1.61	Meuse (P3)	2.51	-
Phthalate	Meuse (P1)	0.96	Meuse (P3)	2.51	*
Phthalate	Meuse (P1-P3)	1.61	Meuse proc. dr.w (P1-P3)	0.54	*

7.1.2 Endocrinic potential and endocrinic disrupting compounds

This paragraph discusses the presence of endocrinic potential and the individual compounds in the investigated samples.

The set of individual results from appendix 1 are summarized in table 7.2. The table shows the ranges of the quantitative results of the ERC bio assay and the individual analytes in March, June and September for the surface water samples.

In the majority of the samples oestrogenic potential could be established. The results vary considerably and do not show a dependency with the time of the season the samples were taken. The results of individual substances show that not every individual compound was detected. Those substances which were detected, oestron and in a single case 17β oestradiol, bisphenol A, phthalates and nonylphenolethoxylates, show a similar pattern: the concentrations vary from non detect to high values for all seasons. However, the data show that the concentrations in March are generally lower than in June and September. The water drainage of the rivers in 1999 was higher in March (Meuse 697 m^3/s; Rhine 4751 m^3/s) than in June (96 m^3/s; 3302 m^3/s) and September (41 m^3/s; 1356 m^3/s). This may indicate that the xeno-oestrogens do occur in the rivers over the whole year and are more diluted in periods with high drainage.

ER-CALUX assay (ERC assay)

The response of the ERC bio assay, which is a measure for the oestrogenic potential, in surface water samples were relatively low. The response of this test has been reported in ng/l EEQ (estradiol equivalents), which relates to the response as if 17β-oestradiol had been determined. All observed concentrations in both Meuse and Rhine were below 0.3 ng/l EEQ. At levels of 0.3 ng/l EEQ and higher, effects on vitellogenin production have been observed in fish living in surface waters (Job 1993 and Har 1999). In this report we use the 0.3 ng/l EEQ as a maximum acceptable level for the preparation of drinking water from surface water. In all cases the measured responses were lower than the maximum acceptable level.

In both rivers there was a response on the ERC test. The median oestrogenic potential in the Meuse was significantly higher than in the Rhine (see table 7.1) when the three phases are compared. The high oestrogenic activity at Eijsden, 0.154 ng/l EEQ (Meuse phase 1, appendix 1) can not be explained by the high water drainage which is the highest in the year. It emphasizes that concentrations may vary considerably over time and shows the need for more frequent measurements in order to obtain more accurate information about the extend of the oestrogenic potential. The variable results of the phthalate and bisphenol A measurements indicate a similar need.

The first evaluation of these data indicates that oestrogenically active compounds sometimes occur in comparable levels in both surface waters and finished drinking waters. The results are illustrated by two figures, fig.7.1 for the Meuse and figure 7.2 for the Rhine.

The remarkably high value of the ERC assay in finished drinking water from the Twentekanaal, 0.201 ng/l EEQ (Rhine phase 2, appendix 1), needs further confirmation. The phthalate and bisphenol A concentrations for instance in the Twentekanaal drinking water (phase 2) were moderate and can not explain this high oestrogen activity. It is remarkable that such a concentration exists in water produced from raw water containing only 0.018 ng/l EEQ, but it can not be excluded. Furthermore from table 7.1 it can be derived that the median values found in surface water in both Meuse and Rhine are significantly reduced during the drinking water production process. The conclusion is that the purification processes are sufficiently effective for the removal of endocrinic disruptors. But on the other hand it does necessarily apply to the situation at the Twentekanaal.

It is further investigated if the measured response of the bio assay can be attributed to the oestrogenic effect of one or more individual substances. It is possible to compare the measured response of the ERC assay at a selection of sampling points with the calculated ERC assay response of the individual chemicals. This exercise is shown in table 7.3. The relative oestrogenic potential factors from table 4.2 in Chapter 4 are used for this purpose. For each sampling point, the samples for the analysis of the ERC assay and the substances were extracted on the same day. The relative oestrogenic potential as determined by the ERC assay response was not known for each component and was consequently not calculated. The table shows that the response of the ERC assay was higher at Eijsden phase 1, Twentekanaal drinking water phase 2 and at Liège phase 1. The individual response of the individual components was far too low to represent the actually measured ERC assay. Apparently, there are more substances responsible for the oestrogenic effect on the biotest as measured directly in the sample. There must obviously be more substances which induce an oestrogenic effect and which are of unknown sort and origin.

In the case of the Brakel surface water in June, phase 2, the sum of the individual expected responses of the analytes determined was considerably higher than the ERC assay suggests. The responses of the chemical and biological methods are based on completely different principles and therefore hard to compare. The biological test is sensitive to

Table 7.2 Overview of the ranges of the quantative results of the biotest (ng/l EEQ) and the individual compounds (ng/l) in surface water

	Meuse			Rhine		
	March	June	September	March	June	September
Waterdrainage m3/s	697	96	41	4751	3302	1356
CALUX-test	0.011-0.034	n.d. - 0.031	n.d.- 0.166	0.007-0.037	0.010 - 0.201	n.d.- 0.045
17-a-oestradiol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17-b-oestradiol	n.d.	n.d.	n.d.	n.d. - 1	n.d.	n.d.
oestron	n.d. - 0.7	n.d. - 4	n.d. - 1.5	n.d. - 2.2	n.d.	n.d. - 0.9
17-a-ethinyloestradiol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
bisfenol A	n.d. - 66	n.d. - 21220	n.d. - 580	n.d. - 1000	n.d. - 150	n.d. - 100
DMP	n.d. - 22	0 - 187	4-105	9 - 36	n.d. - 102	10-37
DEP	0 - 245	0 - 2631	0-2321	n.d. - 52	n.d. - 100	0 - 694
DPP	n.d.	n.d. - 22	n.d.	0 - 11	n.d.	n.d.
DMPP	237 - 646	177 - 2779	0 - 1105	128 - 515	193 - 594	0 - 462
DBP	102 - 461	94 - 1884	67 - 579	60 - 496	211 - 471	0 - 410
BBP	22 - 242	n.d. - 1995	12 - 53	20 - 486	61 - 314	48 - 87
DCHP	n.d.	0 - 61	0 - 8	0 - 20	n.d. - 9	0 - 14
DEHP	181 - 405	219 - 200000	97 - 650	147 - 580	275 - 919	249 - 541
DOP	n.d. - 14	0 - 4644	n.d. - 28	2 - 16	n.d.	n.d. - 16
Total phthalate load	381-1631	236-21220	272-3928	120-186	279-1812	95-3814
nonylfenolethoxylaten	n.d. - 1500	n.d. - 2450	n.d.	n.d. - 4500	n.d. - 2596	n.d.
octylfenolethoxylaten	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
nonylfenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
octylfenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Legend: n.d. = not detected

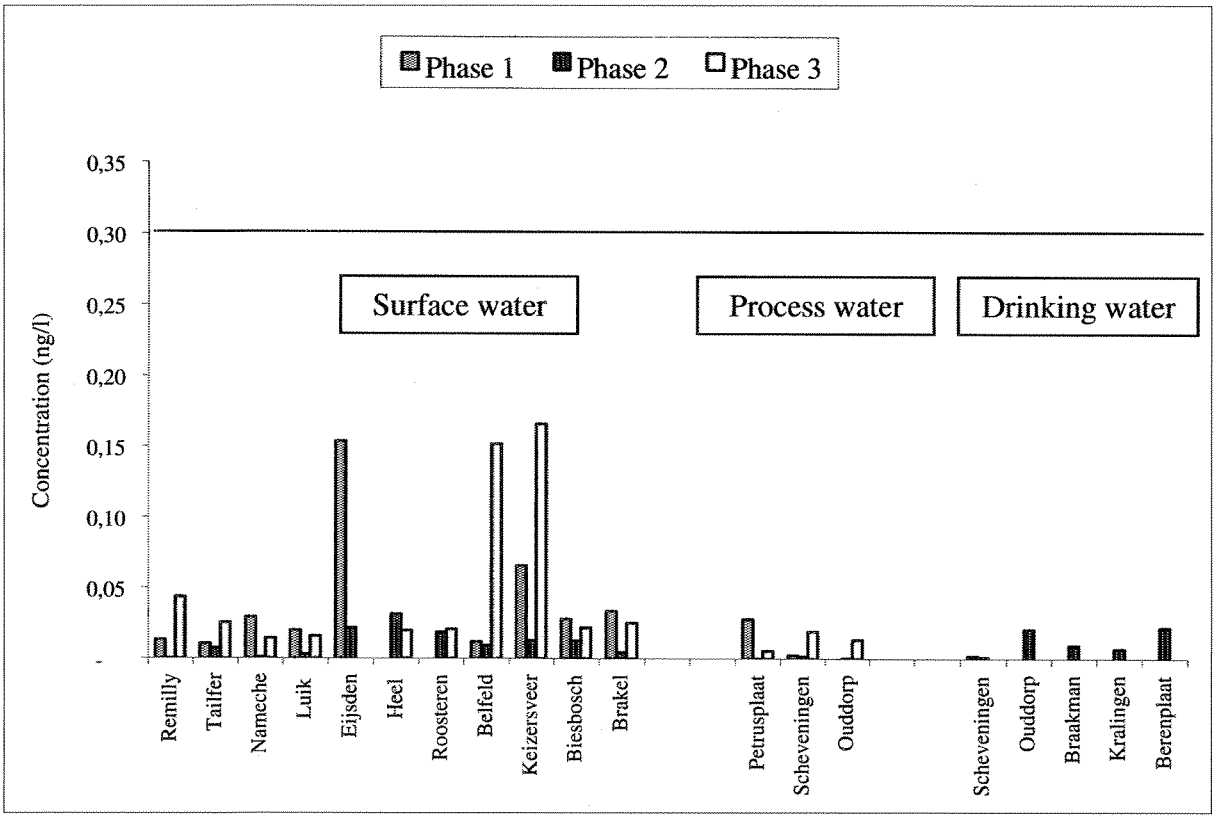

Fig. 7.1 ERC bio assay, oestrogenic activity in the river Meuse, process water and finished drinking water, expressed as EEQ (ng/l); the line at 0.3 ng/l indicates the maximum acceptable level.

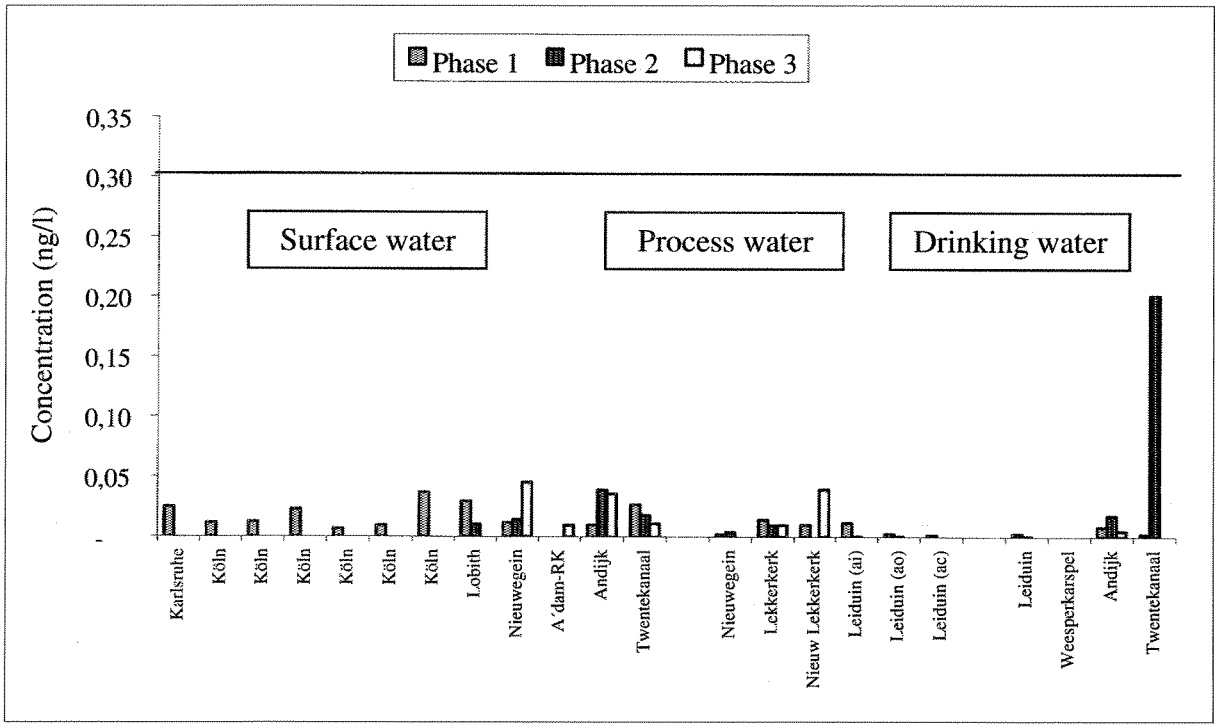

Fig. 7.2 ERC bio assay, oestrogenic activity in the river Rhine, process water and finished drinking water, expressed as EEQ (ng/l); the line at 0.3 ng/l indicates the maximum acceptable level.

Table 7.3 The calculated ER-CALUX response on the basis of individual components versus the determined response in ng/l EEQ

	Eijsden sw phase 1		Twentekanaal dw phase 2		Brakel sw phase 2		Liège sw phase 1	
	Concentration ng/l	ng/l EEQ	Concentration ng/l	ng/l EEQ	Concentration ng/l	ng/l EEQ	Concentration ng/l	ng/l EEQ
date	23-3-99		28-6-99		28-6-99		8-3-99	
17ß-oestradiol	0		0		0		0	
oestron	0		0		0		0	
17α-oestradiol	0		0		0		0	
17ß-ethinyloestradiol	0		0		0		0	
bisphenol A	18	1,40E-04	0		21.220	1,66E-01	66	5,15E-04
dimethylphthalate	0		0		187	2,06E-03	16	1,76E-04
di-ethylphthalate	0		0		2.631	8,42E-05	245	7,84E-06
di-n-butylphthalate	314	5,65E-06	235	4,23E-06	1.884	3,39E-05	276	4,97E-06
butylbenzylphthalate	92	1,29E-04	96	1,34E-04	1.995	2,79E-03	140	1,96E-04
di(2-ethylhexyl)phthalate	283	8,49E-06	290	8,70E-06	200.348	6,01E-03	284	8,52E-06
4-nonylphenol	0		0		0			0
Total expected oestrogene effect		0,0003		0,0001		0,1765		0,0009
CALUX total (measured value)		0,154		0,201		0,005		0

Legend: sw = surface water; dw = drinking water

synergistic and inhibitory effects. The inhibitory effect makes it possible that the combination of substances eventually shows a lower response to the ERC assay.

The calculation and the comparison makes it clear that the chemical and the biological approach give different results. The tests can not replace each other but must be seen as complementary information for the occurrence of oestrogenic potential and xeno-oestrogens. It is, however not possible at this stage to elucidate the oestrogenic potential by the contribution of oestrogenic effect of the individual compounds.

Hormones

The hormones were detected in only a few cases and the concentrations were very low. This is remarkable because in some cases the samples were taken in the vicinity of large cities, such as Liège and Köln. The effluents of sewage treatment works may contain hormones and synthetic hormones which are applied as anti-conceptive. The stability of the hormones 17β-oestradiol and 17α-oestradiol is not very high (see table 4.1), and they degrade for instance to the metabolite oestron. The latter compound was detected in a number of cases although concentrations were rather modest: the highest levels were 2.2 ng/l at site 2 near Köln in March and 4 ng/l in the Meuse.

The synthetic hormone 17α-ethinyloestradiol was not detected in any of the samples. This is not in agreement with values found recently by Belfroid (Bel 1999) who found concentrations at Lobith up to 4.3 ng/l in the autumn of 1997. The number of data points used in 1997 and in the present project was very limited. This means that concentrations can vary and that the time of sampling determines whether the compound will be detected or not.

No traces of hormones were present in drinking water and in process water. As explained above this does not mean that no hormones are present at concentration which show an oestrogenic potential such as in the drinking water sample at Twentekanaal. The detection limit is 0.3 ng/l for the analytical method, but the ERC test responds at much lower concentrations.

Bisphenol A

The values of the analysis are given in fig 7.3 for the Meuse and in fig. 7.4 for the Rhine. The results show that this compound is frequently found in the surface waters of both rivers. The concentrations varied from non-detectable to 22 µg/l in a sample taken in June at Brakel on the Afgedamde Maas.

The median concentration of bisphenol A (see table 7.1) in the Rhine (17.5 µg/l) was not significantly higher than in the Meuse (13.5 µg/l), and the present results indicate a significant reduction during water treatment from 17.5 µg/l to 5.45 µg/l (table 7.1) in the Rhine and from 13.5 µg/l to 5.5 µg/l in the Meuse. This is a comforting thought for the water companies responsible for the purification processes.

The results may provide some indication for the role of industry as a source for this compound. In Germany (Köln), the river was sampled near several factory outlets (see fig. 7.4). The concentrations observed at these sites are distinctly higher than those at Karlsruhe (southern Germany) and in the Dutch part of the Rhine. On the other hand the data are consistently elevated compared to Karlsruhe which may indicate that the before Köln the river was already contaminated. Unfortunately, the number of observations was too low to test these differences statistically. The fact that the concentration decreased in the relatively short distance between Köln and Lobith may indicate a relatively fast disintegration of this compound in the river. This assumption was supported by the Andijk sample point, situated at the rim of Lake IJssel, with an even longer residence time.

A similar pattern could be discerned for the Meuse (fig. 7.3), viz. a strong increase in the industrial area of Liège, followed by a sharp decrease at the Dutch border (Eijsden).

Phases 1 and 3 at the Brakel site showed a low bisphenol A concentration, near the detection limit, but an extremely high concentration was observed (22 µg/l), in the second phase. This coincides with the extreme value of the phthalates at this point, indicating an incidental spill or accident. The source of this accident is not known: it could stem from an industrial source.

Bisphenol A was occasionally found in process water. In the Lekkerkerk and Nieuw Lekkerland samples the concentrations measured during the project were found to be between 30 and 130 ng/l. This water was extracted after being infiltrated in the riverbank. It can be concluded from these results that the source is probably locally contaminated with bisphenol A the whole year round. There are no further data on finished drinking water from this water supply company and further study is advisable.

No bisphenol A was detected in drinking water despite some high concentrations in the source. The chemical was apparently efficiently removed from the raw water during the water purification process: this is confirmed by the significant decrease in the median concentrations shown in table 7.1. as mentioned earlier. It should be noted, however, that the few data which were generated are no guarantee that no bisphenol A will ever be present in drinking water. The relatively high peak concentrations in the source were measured at the same moment as the finished product, which means that the delay time between extraction from the river and the production of the drinking water was not taken into account.

These results are not in good agreement with those of measurements in the period August to December 1997 (Bel 2000). The range of concentration in 1997 at Eijsden and Lobith is considerably higher than in 1999 and varied from 40 – 160 ng/l. In 1999 the concentrations of bisphenol A were between the detection limit and 43 ng/l. These distinct differences show that no accurate estimation of the bisphenol A concentration can be made on a limited number of measurements over such long period of time.

It also indicates that the bisphenol A levels could cause occasional problems for the water purification processes. Bisphenol A is evidently a substance which requires further study at the source as well as in the purification process of the water supply companies.

Phthalates

Of the nine different types of phthalates, dimethylphthalate (DMP), diethylphthalate (DEP), dipropylphthalate (DPP), dimethylpropylphthalate (DMPP), dibutylphthalate (DBP), butylbenzylphthalate (BBP), dicyclohexylphthalate (DCHP), diethylhexylphthalate (DEHP) and di-n-octylphthalate (DOP) only four seemed to be present in higher (>0.1 µg/l) concentrations, i.e. DEP, DMPP, DBP and DEHP in the river Meuse. In the Rhine only two phthalates, DEHP, DBP, occurred in concentrations of >0.1 µg/l. The dominance of individual phthalates differs between the rivers, with DEP being the most important component in the Meuse, followed by DMPP, DEHP and DBP, while the most important phthalate in the Rhine was DEHP, followed by DBP. These differences may be caused by different sort of industries. In Germany samples were taken in the industrial area of Köln, at the outlets of several factories. The results do not indicate a distinct influence of these factories since the concentrations were not distinctly higher than in other stretches of the river in the Köln area. Thus although the source of phthalates is probably industrial, the present study did not pinpoint any particular industry (see fig.7.6).

The difference between the two rivers was also reflected in the total concentration of these compounds (table 7.2), with concentrations in the river Meuse (range 0.2 - 214 µg/l)

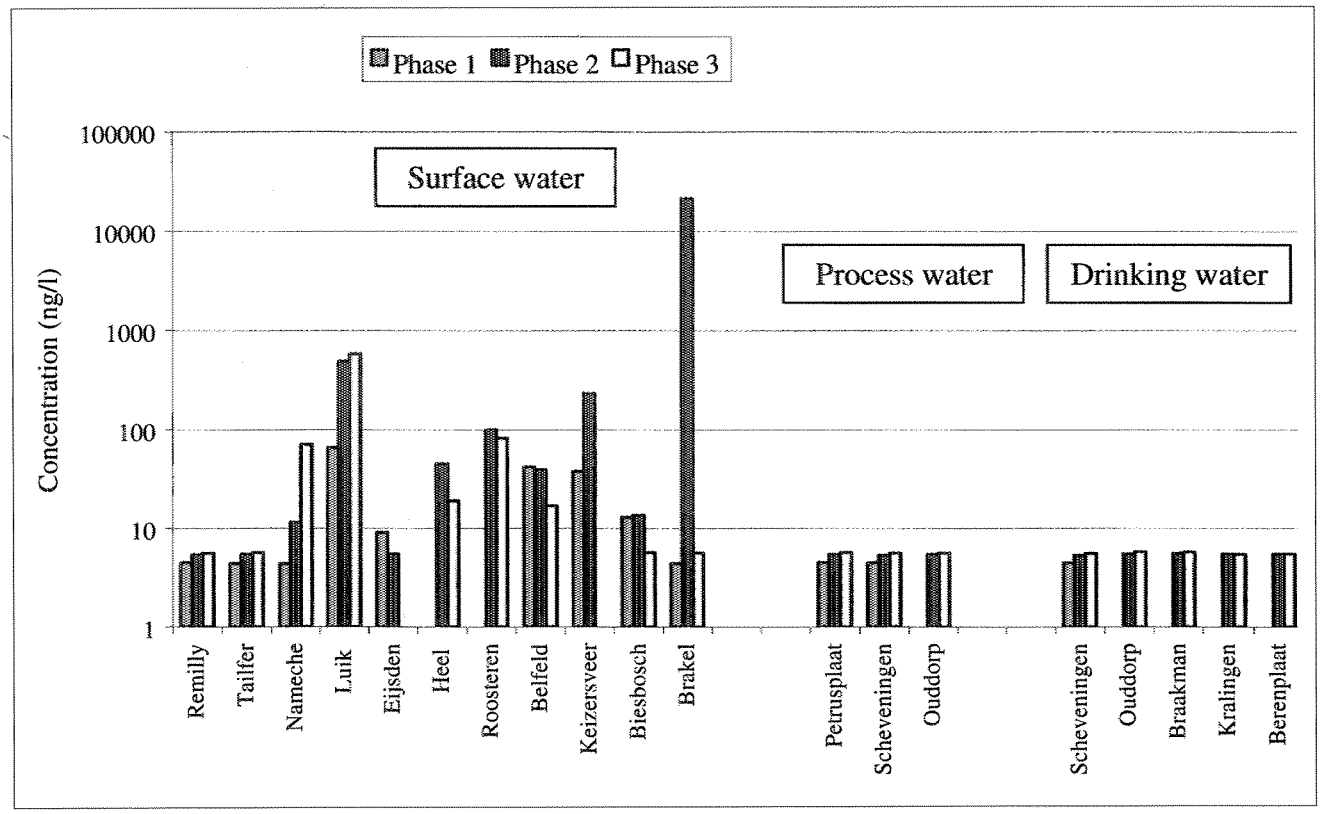

Fig. 7.3 Bisphenol A concentrations (ng/l) at various sampling sites in the river Meuse and at water-works along that river.

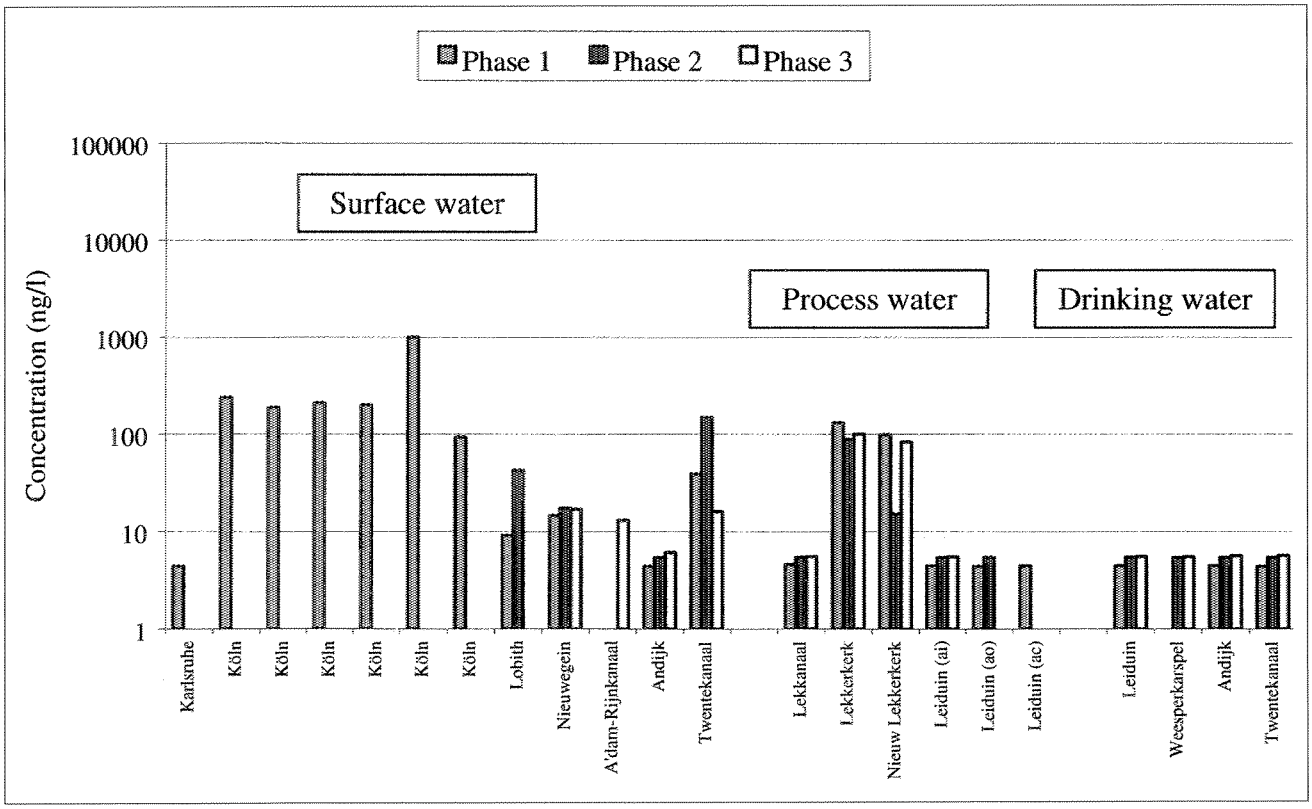

Fig. 7.4 Bisphenol A concentrations (ng/l) at various sampling sites in the river Rhine and at water-works along that river.

being significantly higher than those in the Rhine (range 0.1 - 4 µg/l). This is statistically confirmed by the Mann-Whitney-U test in table 7.1.

Different sample dates (phases 1, 2 and 3) showed different results (Fig. 7.5). These differences, especially in the river Meuse, may be correlated with the time of the year and the water drainage. The water drainage in September is particularly low (41 m^3/s) and concentrations can go up accordingly. The concentrations of phthalates in this season are significantly higher than in March but not significantly higher than in June. At most sampling sites, the phthalate concentration was higher in phase 3. The highest value, however, was observed at Brakel, an oxbow lake where a total concentration of 214 µg/l was observed in phase 2 (DEHP was responsible for 200 µg/l). This extremely high value may have been caused by contamination with substances used in the nearby area combined with the other disadvantageous factors.

The finding at Brakel suggested a close correlation between the occurrence of bisphenol A and that of phthalates. This suggestion was tested statistically for all data points, including the Brakel data. There appeared to be a significant relation of $r = 0.64$ with $r_{(crit.\ 0.05)} = 0.306$ and for $n = 28$. When the aberrant Brakel data were left out, no significant correlation between these two chemicals remained with $r = 0.193$. Therefore this suggestion is not confirmed.

The median concentrations of phthalates in drinking water were significantly lower than those in surface water (table 7.1). However, the purification process producing drinking water from surface water probably does not completely remove phthalates from the water, since the finished product appeared to be contaminated with these substances. The present results do not allow us to decide whether these substances are incompletely removed during the treatment process or are added during treatment. It is known that the use of plastics in the installations can generate this effect. For instance, PVC tubing used for water transport releases phthalates by leaching into the water (Jun 1974).

It can probably be ruled out that the higher values observed during the third phase in the river Meuse are analytical artifacts since `normal` values were obtained in the river Rhine series in the same period. The total phthalate concentration varied between different sample points. In the river Meuse high concentrations were observed particularly in the second and the third phase. The fact that these phases in the Rhine did not show distinct differences leads to the conclusion that the observed fluctuations are not due to analytical errors (fig. 7.5 and 7.6). Unfortunately only these series of measurements are available, these cannot be related to seasonal fluctuations or any pollution pattern. Frequent measurement, for instance at a limited number of sampling sites are necessary to determine seasonal variation.

The high phthalate variation observed during the third phase is in finished drinking water indicates that these compounds may break through in the purification process. Leaching from drinking water materials is improbable since the high concentrations occur only during the third phase in the river Meuse area.

The majority of the results of this study are in agreement with the data by Belfroid et al. (Bel 2000) who collected samples of surface and drinking water in the Netherlands in the autumn of 1997. The authors found concentrations of individual compounds not exceeding 2.6 µg/l. In the project reported here the concentrations in surface water ranged from the detection limit to 5 µg/l for individual compounds, with one exception earlier mentioned: in a sample obtained at Brakel the concentration of di(2-ethylhexyl) phthalate (DEHP), was 200 µg/l, which is very high compared to the other samples.

The 1997 data on drinking water differ little from the present findings. The concentrations measured in 1997 ranged from below the detection limit to 3.5 µg/l, and were slightly higher than the majority of those measured last year (< d.l. to 2.1).

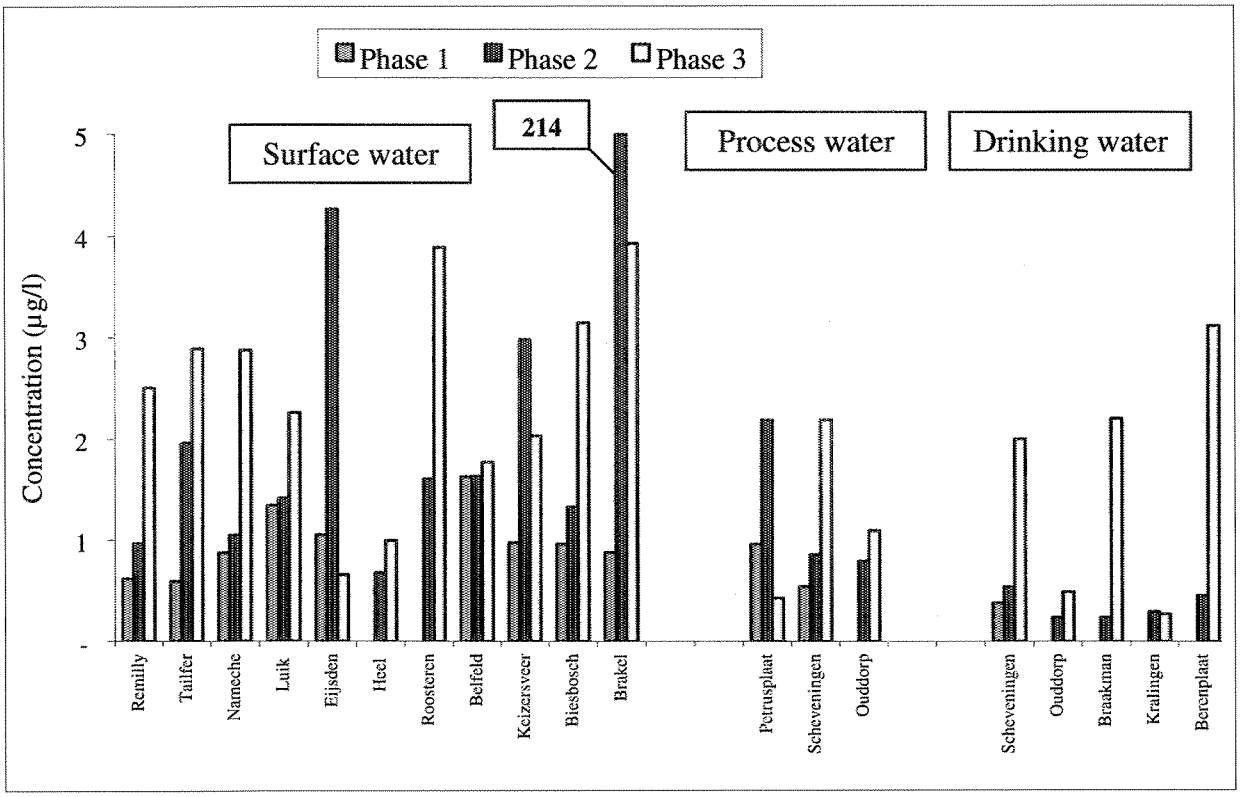

Fig. 7.5 The total phthalate concentration at various sampling points in the river Meuse and process and drinking water of waterworks along the river.

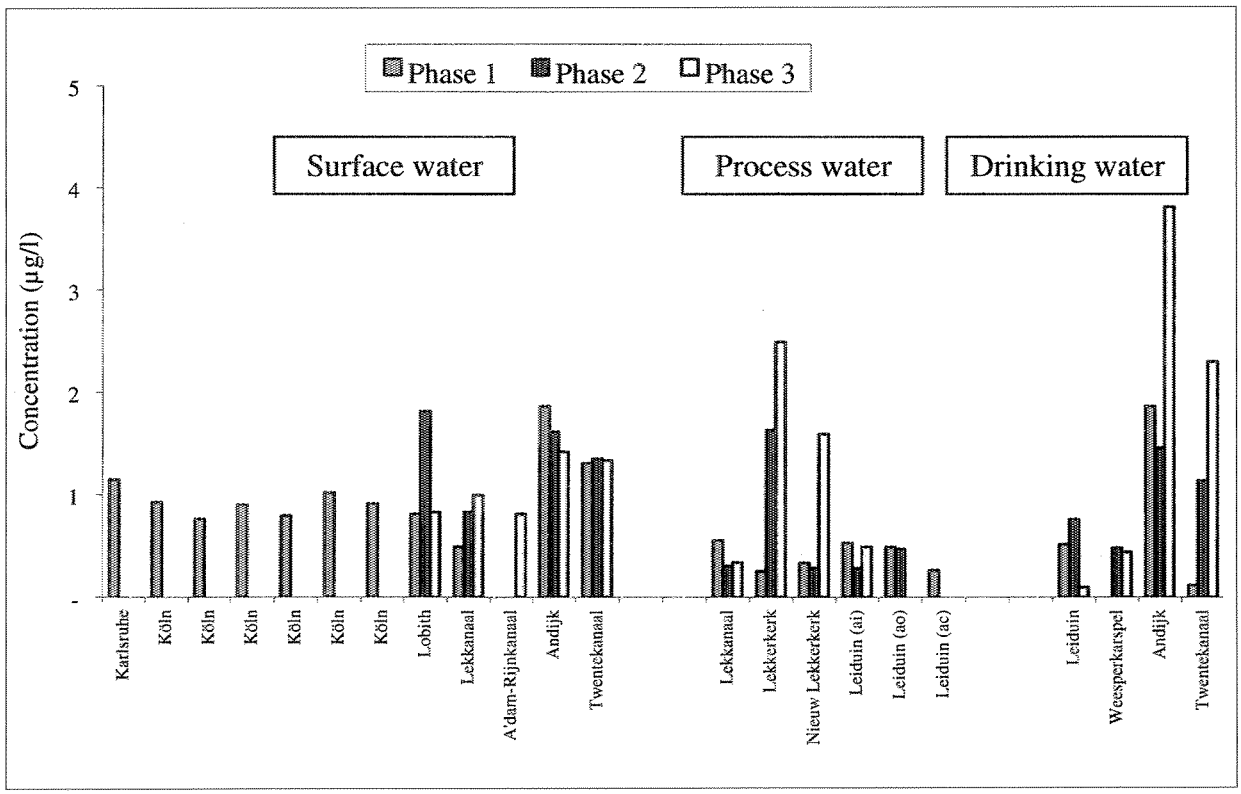

Fig. 7.6 The total phthalate concentration at various sampling points in the river Rhine and process and drinking water of waterworks along the river.

Alkylphenol polyethoxylates
The presence of alkylphenol polyethoxylates and their metabolites was only occasionally detected: this was particularly the case for nonylphenolethoxylate. In June, nonylphenolethoxylate was present in the Meuse at Roosteren and at Belfeld, in concentrations of 2.5 and 2.0 µg/l respectively. In the Rhine, this substance was found at Lobith (2.6 ng/l) and at Nieuwegein (1.9 ng/l), also in June.

In two individual cases, nonylphenolethoxylates were found in process water. In Scheveningen in March, after extraction of dune-infiltrated water (1.5 µg/l), while no nonylphenolethoxylates were found in other Meuse samples. In Nieuw Lekkerland in June the concentration was 1.6 µg/l. In this case nonylphenolethoxylates were also detected in the same period at Lobith and at Nieuwegein. It may be possible that a pollution in the Rhine caused the presence of this substance in extracted water of Nieuw Lekkerland after river embankment infiltration.

In June nonylphenolethoxylate was found at Kralingen and at Berenplaat. The concentrations observed were 2.1 µg/l for both sampling sites. Nonylphenolethoxylates were present in Meuse water at Roosteren and Belfeld. It is not plausible that this observation has a connection with the result of the Rotterdam samples since the delay time is considerable between the sampling points.

In March, the polymer was also present in drinking water at Andijk, in a concentration of 2 µg/l. No other sample of the Rhine contained detectable traces of the substance during that period.

Nonylphenol was not present in the samples, which is not in agreement with observations made some years ago, when the substance was detected in some surface water samples (Bel 2000). In this article the substance nonylphenolethoxylate was not found in surface water, while significant concentrations were found in sediment .

From the results it can be concluded that the variation is considerable and that an increased number of data points is necessary in order to give a better insight in the actual concentrations of these substances.

7.1.3 Quantitative evaluation of the results

The investigations for the LOES-project concentrated on a relative large number of sampling sites. These sampling points were sampled only three times, several even less due to changes of the sites during the project. The required number of samples can be determined by considering some aspects of the river. Sanders *et al.* (San 1994) give a diagram from which the numbers of sampling stations and numbers of samples can be read, based on the drainage area, the velocity of the water and the ratio between maximum water flow and minimum flow. For the River Rhine this ratio is only 3,5 and for the River Meuse 20, with drainage areas of 185000 km^2 and 36000 km^2, respectively, at least 10-12 measurements, for both rivers, are necessary for a reliable monitoring.

Once a number of samples have been analysed, the number (n) of samples necessary for reliable estimations (95% interval) can be calculated from the variables sample mean (x) and standard deviation (σ) in a normal distributed or log-transformed population (San 1994).

$$x - 1.96(\sigma/\sqrt{n}),\ x + 1.96(\sigma.\sqrt{n})$$

This calculation is based on the estimation of confidence interval, which have been calculated for some of the parameters measured here (table 7.4).

The formula for calculation of confidence intervals can be used to estimate the number of samples necessary for obtaining results within a defined confidence interval (San, 1994). The desired 95% confidence interval (I) for a normal distributed constant concentration in a series of independent samples is equal to:

$$I = 2 * (1,96) * \sigma/\sqrt{n}$$

For instance if one wishes to determine with 95% certainty the range within one standard deviation from the mean concentration, 15.4 samples are necessary. If a wider range is accepted, for instance two times the standard deviation from the mean concentration, only 3.8 samples are required. This example shows the degree of uncertainty in the data obtained in this study. In future studies it is advisable to investigate between 4 and 15 samples.

Table 7.4 The 95% confidence intervals for some of the variables measured in this study, based on not log-transformed data; n is the number of measurements. The phthalates are tested as the total sum of all individual compounds.

location	variable	minimum	maximum	unit	n
Eijsden	phthalates	0,00	4,23	µg/l	3
Lobith	phthalates	0,50	1,80	µg/l	3
Eijsden	bisphenol A	5,45	8,65	ng/l	2
Lobith	bisphenol A	15,62	23,94	ng/l	2
Eijsden	ERC	0,000	0,217	ng/l	2
Lobith	ERC	0,001	0,039	ng/l	2

A remarkable difference between Liège and Eijsden concerning the bisphenol A concentration has been observed. The 95%-confidence intervals are respectively 265 ± 3,7 and 7.0 ± 2,0 ng/l and indicate a significant difference. Based on only a few data these differences are not easily explained. Since Liège and Eijsden are only a few kilometres from each other, these differences may indicate a rapid disintegration of bisphenol A in the river. Dilution is not very probable since no important streams join the Meuse between both sampling stations, unless the Liège samples were taken in the plume of a polluting factory outlet. The fact that the Liège samples have always (n = 3) a high concentration and Eijsden was low on both sampling days, make a difference based on hazard improbable. The probably complicated dynamics of pollution, dilution and disintegration of the studied compounds can only be elucidated with more intensive sampling programs. Knowledge on the disintegration time is important for the estimation of the importance of a certain compound and also for detection of its polluting source along the river. Evaluation of changes of concentrations along each river is still difficult, due to a relatively low number of data. Especially information on the dynamics of these compounds is needed before detailed evaluation can be made. In a river with a more or less constant concentration of a compound, four to six samples per year may be enough to establish the mean yearly load in that river. But as soon as strong variation, dilution or disintegration dynamics of the compounds seem to play an important role, more samples per year are needed. A monthly sampling program will give relative reliable results. Matters as disintegration of compounds cannot be studied efficiently in normal monitoring programs. These aspects are best studied in special designed programs, such as monitoring in fliessende Welle, where a certain amount of polluted water is surveyed over a longer time to establish the stability of such a compound in the river. Rapid

disintegration is probably an aspect for substances showing large differences in concentration over only short distances in the river, compounds such as bisphenol A at Liège and Eijsden may be appropriate for a special stability study.

7.2 Risk assessment of the endocrine disruptors found

Risk assessment of endocrine disruptors depends on their determined concentrations, on the chemical and biophysical properties and on the individual toxicological potential of the product. In this paragraph the risk of the individual endocrinic compounds is discussed. This is done for the substances which have been detected in surface water and for those substances which were detected in some occasions in drinking water.

The Actually Determined Concentration (ADC) is the basis of this assessment and replaces the Predicted Environmental Concentration (PEC). This value represents the concentration to which aquatic species are in fact exposed. The concentration found in the water sample is divided by the No Observed Effect Concentration (NOEC). This is the concentration at which no endocrinic disrupting effect on organisms has been reported in preferably *in vitro* studies such as the test on vitellogenine in trout (Job 1996). In the case that those studies were not available the estrogenic equivalent factor (EEF) of the ERC assay was used (Leg 1999) as shown in table 4.2. If the ADC divided by NOEC is smaller than one, no effect should be expected. However, due to synergism, long exposure times and exposure during varies lifecycle stages of aquatic species the endocrinic disrupting effect may take place at much lower concentrations. For this reason the National Health Council in the Netherlands uses a safety factor of 100 to ensure the toxicological safety of the aquatic environment. Subsequently, the quotient of ADC and NOEC should be < 0.01 to ensure a toxicologically safe water (Gez 1999). This criterion is used here as a standard for the toxicological risks of human beings with the simple and straight argument that water safe for the aquatic ecology is safe for the preparation of drinking water with conventional techniques such as river embankment infiltration or dune infiltration.

A synoptic table (table 7.5) summarizes the data. In this table the ADC/NOEC is calculated for the maximum concentrations per compound in the Meuse and in the Rhine. For the ease of the discussion we assume that the maximum concentrations which were found in the Meuse and the Rhine can reach the extraction points of the water companies and therefore the concentrations of the individual substances are representative for the possible risks for the production of drinking water. In table 7.5 the quotient of ADC and NOEC is in the majority $>$ 0.01.

Hormones. The hormones were only occasionally detected in surface water. The concentration are near the detection limits and seem very low, however, the NOEC data are in a number of cases equal or even lower. Consequently the quotient of ADC and NOEC exceeds 0.01 and no guarantee can be given that hormones are not present at the concentration of < 0.001 ng/l. This means that one must be aware of the risk of using surface water for drinking water production despite the fact the substances were not detected.

The NOEC of 17β-oestradiol is 1 ng/l and the detection limit is 0.8 ng/l. The compound was detected in the Rhine at a concentration of 1 ng/l. The quotient of ADC and NOEC is 1 and therefore the criterion of 0.01 is amply exceeded. This is also the case for the non-detects in the Meuse; with a detection limit of 0.8 ng/l the quotient ADC/NOEC can still be larger than 0.01. The limitation of the analytical method can not exclude that relevant concentrations of this undesirable compound are still present in water. The same argumentation is valid for 17α-ethinyloestradiol. A concentration of 0.1 ng/l of 17α-ethinyloestradiol leads in water to the production of vitellogenin in male Rainbow trout

(Pur 1994). This value is lower than the detection limit of the analytical method. A reported figure of lower than the detection limit will not automatically mean that the water is safe for drinking water production since the ADC/NOEC can be between 0.01 and 3. The maximum values for oestron were 4 and 2 ng/l in the Meuse and Rhine respectively. The resulting ADC/NOEC values are 0.160 and 0.88, largely above the criterion of 0.01.
The hormones have moderate log K_{ow} values between 3 and 4, and the half live of the compounds vary between 12 and 30 days. It can not be excluded that the mobility of the hormones is such that a simple purification process is not appropiate for the adequate removal. Moreover the high detection limits of the analytical method compared to the NOEC values will not exclude the presence of hormones at an undesirable concentration level in drinking water. This unsatisfactory situation makes it necessary that methods will be developed in the future with lower detection limits for the determination of hormones.

Bisphenol A. In the literature two NOEC values have been found for bisphenol A. Staples et al (Sta 2000) determined a toxicological NOEC of 64 µg/l in three aquatic organisms. A more specific oestrogenic NOEC value was determined by Broton (Bro 1995) with an E-screen *in vitro* test. The reported maximum concentrations of bisphenol A are high in Meuse (21.2 µg/l) and Rhine (1000 µg/l) and consequently the ADC/NOEC criterion is largely exceeded. The ADC/NOEC values vary from 0.03 to 435.
Bisphenol has a log K_{ow} of little over 3; the DT_{50} is not known. In process water of the WZHO at the extraction points at Lekkerkerk and Nieuw Lekkerland bisphenol A was found in each water sample during the three phase 1 - 3 in concentrations varying between 83 and 130 ng/l. This finding indicates that river embankment infiltration will not be effective in removing this compound and that the mobility of bisphenol is such that it will break through simple purification barriers. In those cases bisphenol A may be a risk for drinking water production. On the other hand, table 7.1 shows the general picture that the concentration of bisphenol A was significantly reduced in all purification processes of the water companies in the Meuse and Rhine basin when compared to the concentration in surface water.

Phthalates. The majority of the NOEC values of the phthalates were mostly calculated from the oestrogen equivalent factors in table 4.2 (see chapter 4.2) which are related to the ERC bio assay. The criterion of 0.01 was only exceeded in the case of dimethylphthalate and di-(2-ethylhexyl)phthalate. Dimethylphthalate (DMP) had a ADC/NOEC value in the Meuse of only 0.02, just above the criterion. The ADC/NOEC of di-(2-ethylhexyl)phthalate (DEHP), which has a general toxicological NOEC, was high for the Meuse(2.778) and just slightly above 0.01 for the Rhine (0.013).
The log K_{ow} of the phthalates vary between 1.6 and 9.6 and the DT_{50} between 3 and 250 days. The phthalates may break through the purification processes. This is shown in the results of the process and drinking water samples where various phthalates were detected. The concentration range is between 0.013 and 0.646 µg/l for DMP and DEHP. Other phthalates are also present in drinking water allthough the concentration levels are very modest and the ADC/NOEC criterion is met (below 0.01). The purification processes in the Meuse basin remove the phthalates significantly (table 7.1) from surface water. These observations make it probable that phthalates do not form a risk for the drinking water production. In one case however, a peak concentration of 200 µg/l DEHP was measured at Brakel in the Meuse in September. Since the delay time is not accounted for (samples of surface water and drinking water were taken at the same moment) further study should be considered.

Table 7.5 The actual determined concentrations (ADC) replaces the predicted environmental concentration (PEC) divided by the No Observed Effect Concentration (NOEC) in surface water. The quotient should not exceed 0.01.

			Meuse		Rhine	
	N(O)EC (µg/l)	Ref.	max conc. (µg/l)	ADC/N(O)EC	max conc. (µg/l)	ADC/N(O)EC
17ß-oestradiol	0.0003	Rou 1998	< 0.0008	< 2.7	0.001	3
oestron	0.025	Tyl 1998	0.004	0.16	0.002	0.088
17α-oestradiol			< 0.0003		< 0.0003	
17α-ethinyloestradiol	0.0001	Pur 1994	< 0.0003	< 3	< 0.0003	< 3
bisphenol A	64.0	Sta 2000	21.2	0.33	1000	15.6
	2.3	Bro 1995	21.2	9.2	1000	434.8
dimethylphthalate	27.3	Leg 1999[1)]	0.2	0.02	0.1	< 0.01
di-ethylphthalate	9.4×10^3	Leg 1999	2.6	< 0.01	0.7	< 0.01
dipropylphthalate			0.0		0.0	
dimethylpropylphthalate			2.8		0.5	
di-n-butylphthalate	1.7×10^4	Leg 1999	1.9	< 0.01	0.5	< 0.01
butylbenzylphthalate	213	Leg 1999	2.0	< 0.01	0.5	< 0.01
dicyclohexylphthalate			0.1		0.02	
di(2-ethylhexyl)phthalate	72	Slo 1990	200.0	2.8	0.9	0.013
di-n-octylphthalate	1×10^4	Leg 1999	4.6	< 0.01	0.02	< 0.01
nonylphenolethoxylates	100	Rou 1996	2.5	0.025	2.5	0.025
octylphenolethoxylates			< 0.9		< 0.7	
4-nonylphenol	10	Job 1996	< 1.5	< 0.15	< 0.6	< 0.06
4-octylphenol	5	Job 1996	< 0.5	< 0.1	< 0.3	< 0.06

[1)]The values with the reference Leg 1999 are calculated values.

Alkylphenol polyethoxylates. Nonylphenolethoxylates were present in Meuse and Rhine at concentrations upto 2.5 µg/l. The NOEC of this substance is 100 and ADC/NOEC's are 0.025 in both rivers and thus higher than the criterion of 0.01. The alkylphenols 4-nonylphenol and 4-octylphenol were not detected. However, the detection limits are relatively high and therefore the ADC/NOEC quotients exceed the criterion of 0.01. Consequently, the alkylphenols may be present at concentrations which may have adverse endocrinic effects.

In process water the maximum concentration of nonylphenolethoxylates is 4.5 µg/l (Nieuwegein, finished product, March). In drinking water the substance was also present and the maximum concentrations was 2.1 µg/l (Kralingen and Berenplaat, June). These concentrations are higher than advisable when the ADC/NOEC criterion is applied.

The majority of the studied compounds were detected in the river Rhine and the river Meuse at concentrations which may induce endocrinic disrupting effects. From this point of view these compounds form a risk for the production of drinking water from Rhine and Meuse water. After purification by river embankment infiltration and dune infiltration, it is to be expected that the mobility of bisphenol A, phthalates and nonylphenolethoxylates is such, that these compounds may break through the purification process. This has been confirmed in the present study by the presence of these compounds in process and drinking water. The concentrations found in drinking water meet the safety criterion of the Dutch Health Council (ADC/NOEC < 0.01).

However, we have shown that high concentrations of the bisphenol A and phthalates can be present in surface water. And thus, monitoring remains advisable.

As far as hormones and alkylphenols are concerned, no clear conclusions can be drawn. The NOEC concentrations of these substances are lower than the detection limits of the analytical methods. Consequently, no guarantee can be given that hormones and alkylphenols are not present at concentration levels which do have adverse effects.

Substances with a low log K_{ow} (< 3) will possibly break through sand filters and active coal filters and eventually be found in drinking water.

The BCF or bioconcentration factor is a measure which accounts for the storage in living organisms and ultimately determines the bioavailability of the product in the organism and at the oestrogenic receptors.

Substances with a profile of high ADC/NOEC, high persistence in water, moderate log K_{ow} and relatively large BCF (see Table 4.1) are compounds which may constitute a threat to the quality of the drinking water production. The actual risk largely depends on the production process involved. The main factor, which could be determined for a number of compounds in the present study, is ADC/NOEC.

Table 7.5 shows that hormones represent a problem when they are detected. At concentrations near the detection limit this is already the case, although fortunately only in very few samples. No NOEC data for bisphenol A are available, but the present concentrations in Meuse and Rhine speak for themselves and are a reason for concern.

Some of the phthalates exceeded the limit of 0.01 ADC/NOEC. Di(2-ethylhexyl)phthalate (DEHP) had a value of 2.778 in the Meuse and of 0.013 in the Rhine, while di-n-butylphthalate had a value of 0.022 in the Meuse. DEHP is very persistent and has a high BCF, so its presence warrants further study.

7.3 Occurrence of endocrine disruptors in the drinking water processes

This chapter surveys the situation in the individual processes used by the water supply companies. We have already reported our preliminary conclusion on the basis of the available data, by saying that the purification processes are generally quite effective. However, we want to emphasize here that much remains to be done before this conclusion can be confirmed.

Figures 7.7 to 7.10 show the results of the analysis from the source to finished product as far as data are available. In fact this is the cross-section of the individual purification processes of the water companies.

The Meuse water supply companies

We have seen that the contamination of the Meuse by endocrine disrupting compounds is more serious than that in the Rhine. This is shown by the higher values of the ERC test. Consequently, the purification processes used by these companies to deliver drinking water of satisfactory quality must be more robust. Figure 7.7 provides a cross-section of the purification process regarding bisphenol A concentrations. A similar cross-section is shown in figure 7.9 for the total phthalate concentration.

At DZH's Brakel inlet, high concentrations of phthalates - 214 µg/l - and bisphenol A - 21 µg/l - were observed in June. At the same time, samples taken after dune infiltration (Scheveningen pw) and filtration (Scheveningen dw), were found to be relatively clean. The samples were collected on the same day and the delay between extraction from the source and the production of drinking water was not taken into account. Thus it is not certain that the production process efficiently removes such peak concentrations of contaminants.

The raw water supply for WBE in Rotterdam is produced by WBB at the Biesbosch area. Water extracted from the Meuse is stored in a water reservoir over a number of weeks. Subsequently the raw water is transported to WBE's production facilities. The process applied at these facilities achieved efficient removal of bisphenol A. Phthalates, of which higher levels were found at Keizersveer and at the Biesbosch, were already partly eliminated in the water basins at Petrusplaat. The subsequent stages of the process effectively removed any remaining phthalates from the drinking water at Kralingen, the facility which receives its water from Petrusplaat. At Berenplaat, the drinking water is also prepared from Petrusplaat water.

The origin of the phthalates found in September in a sample of Berenplaat is not clear; they were only found in this sample.

The source for the drinking water production by Delta Nuts at Braakman is also the raw water produced by WBB. At the Braakman production facility, the water is treated by ozonization and subsequently filtered through active coal. A slightly increased level of phthalates was found in September of which the origin is not known.

The production at Ouddorp uses surface water from the Meuse and Rhine extracted from the Haringvliet. The concentrations of the chemicals were comparable to those measured in samples from Keizersveer. Bisphenol A was found to be adequately removed after dune infiltration (Ouddorp process water), and phthalates could no longer be detected after the last purification steps in the process, with active coal filtration and UV desinfection (Ouddorp drinking water).

Along the Meuse near Liège, urban and industrial effluents produce considerable concentrations of oestrogenic compounds over the year (see fig. 7.3 and 7.5). At Eijsden several increased concentrations have been observed (see fig.7.1 and fig.7.7). Water companies which eventually use water from the vicinity of these points should be interested in an increased monitoring of the sampling points at Liège and Eijsden. This is confirmed by the results at the WML production plants at Heel and Roosteren. Bisphenol A concentrations

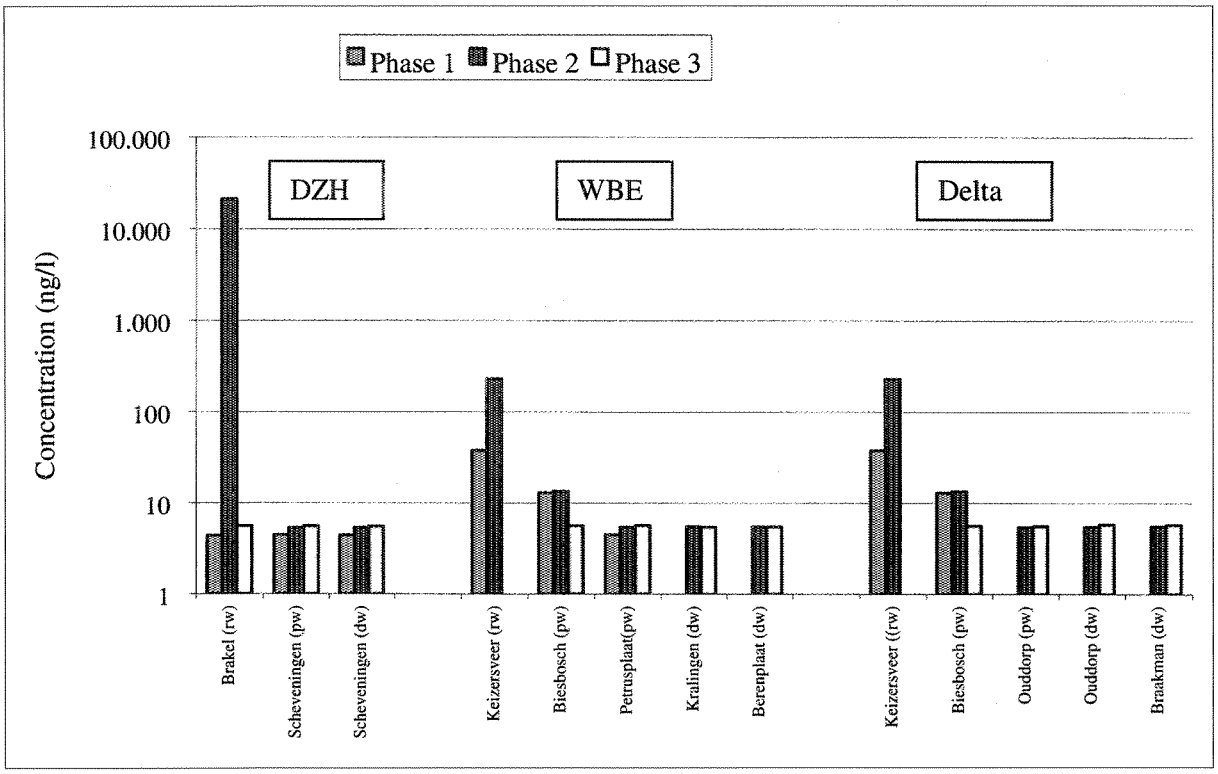

Fig. 7.7 The Bisphenol A load in raw water (rw), process water (pw) and in finished drinking water (dw), prepared from Meuse water.

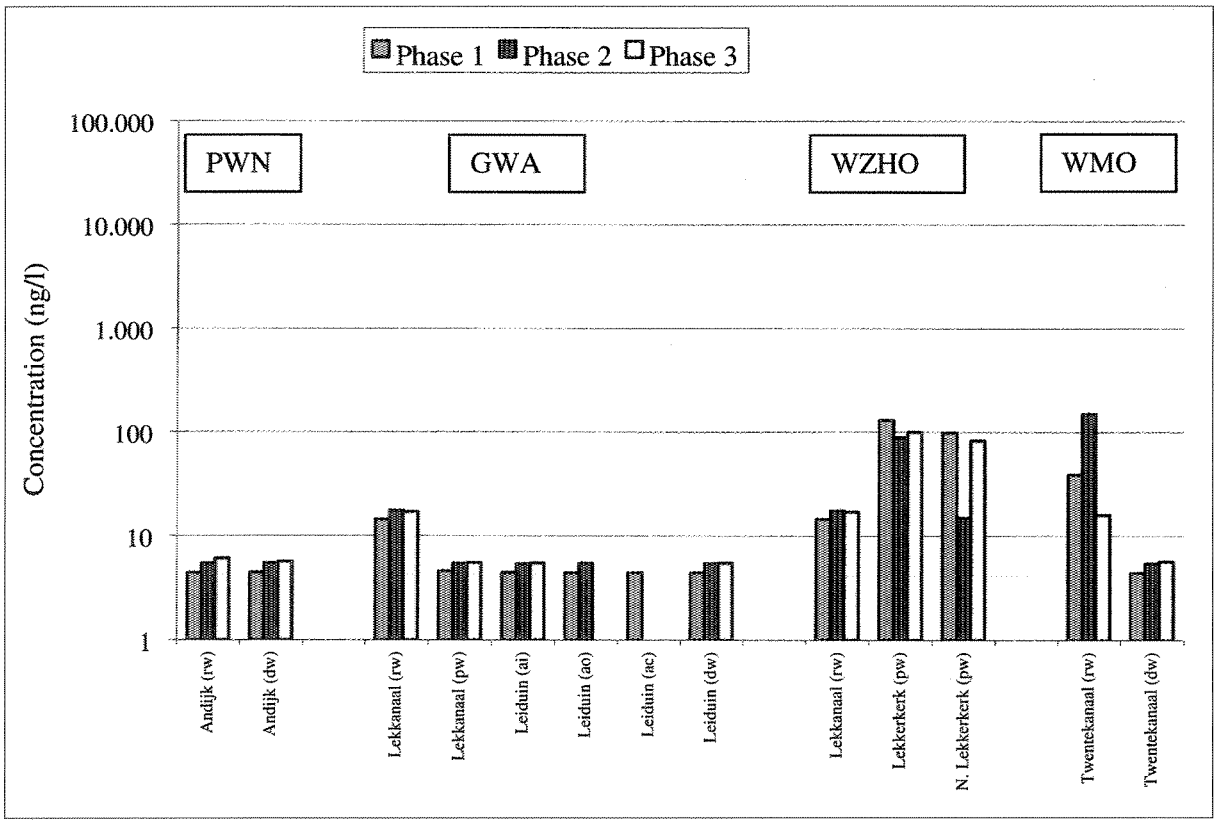

Fig. 7.8 The Bisphenol A load in raw water (rw), process water (pw) and in finished drinking water (dw), prepared from Rhine water.

were increased in June and September (see fig. 7.3) and in September the total phthalate concentration was also increased.

The Rhine water supply companies

The water supply companies along the Rhine presumably have a lighter task in purifying surface water from the endocrine disrupting chemicals which were assessed in the present study. Concentrations of bisphenol A and total concentrations of phthalates were restricted to modest concentration levels. The bisphenol A concentration was approximately 0.1 µg/l and the total concentration of the phthalates did not exceed 4 µg/l.

The geographic situation of the production plant at Andijk again proves its favorable position for bisphenol A. Concentrations of bisphenol A were low and the removal of this substance can be guaranteed. The phthalate concentration, however, was higher than that faced by the other companies and the purification process did not seem to be able to remove this type of compounds. Another possibility is that the water is recontaminated during the process. The use of polymer tubing in the installation may be responsible for this effect. This could be the case particularly in the September sample (phase 3), when a concentration of 4 µg/l was found in the drinking water sample.

The GWA production facilities at Leiduin and Weesperkarspel adequately removed the low traces of the compounds studied. The sources and the raw waters were relatively clean and the purification process completely removed the analytes.

The situation at WZHO has also been plotted in the figures 7.8 and 7.10. Their process water is extracted after bank infiltration of river water. The concentration of bisphenol A varied from 30 to 130 ng/l in extracted water in Lekkerkerk and Nieuw Lekkerland. This is remarkably high when compared with the reference point at the Lekkanaal. The difference may be due to a local spill between the two points along the river.

There are no data available on drinking water produced by WZHO. It is interesting to study further the presence of bisphenol A in drinking water, in order to assess the possible risk of the drinking water production by riverbank filtration.

The WMO water supply company extracts water from the Twentekanaal for the production of drinking water. Bisphenol A was found in increased concentrations in the Twentekanaal. The production process is such that elimination of bisphenol A was found to be satisfactory. In the case of phthalates, removal can be efficient, as is shown by the March data. In June, however, no elimination took place and in September the concentration in drinking water was even higher than in surface water. There is no satisfying explanation for this phenomenon which should be further studied.

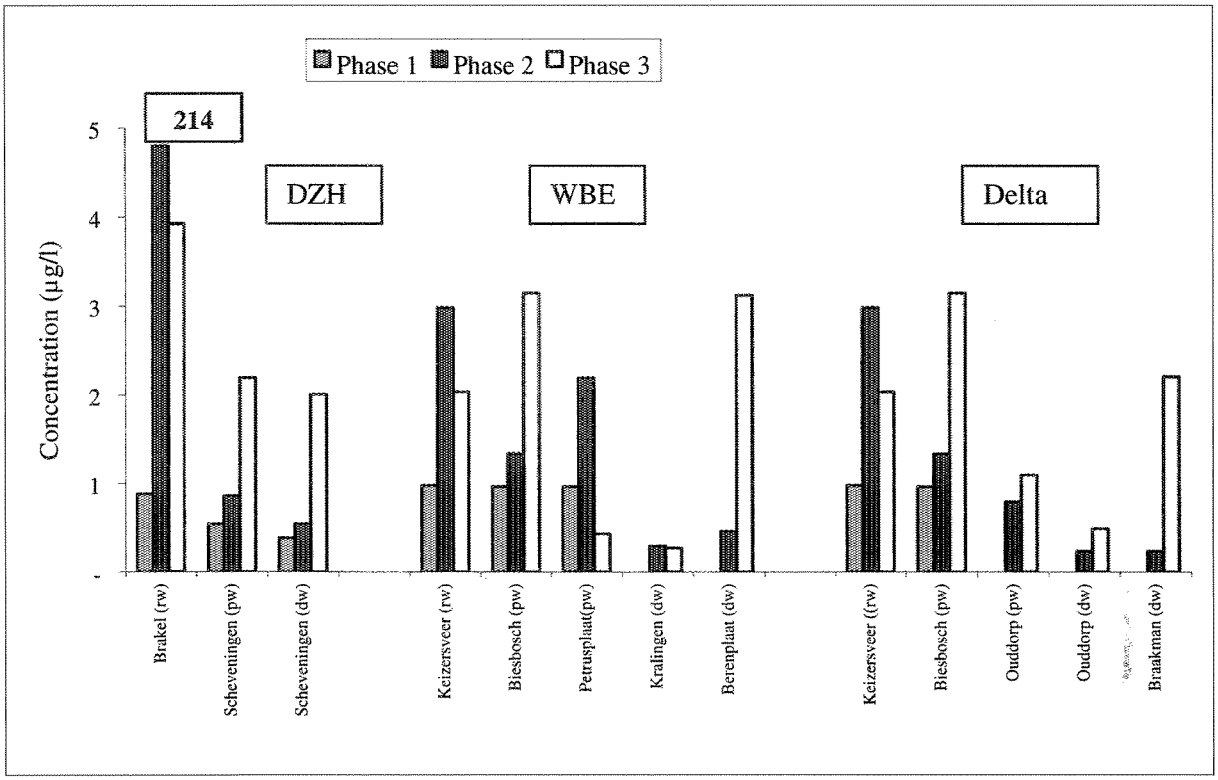

Fig 7.9 The total phthalate load in raw water (rw), process water (pw) and in finished water (dw), prepared from water of the Meuse.

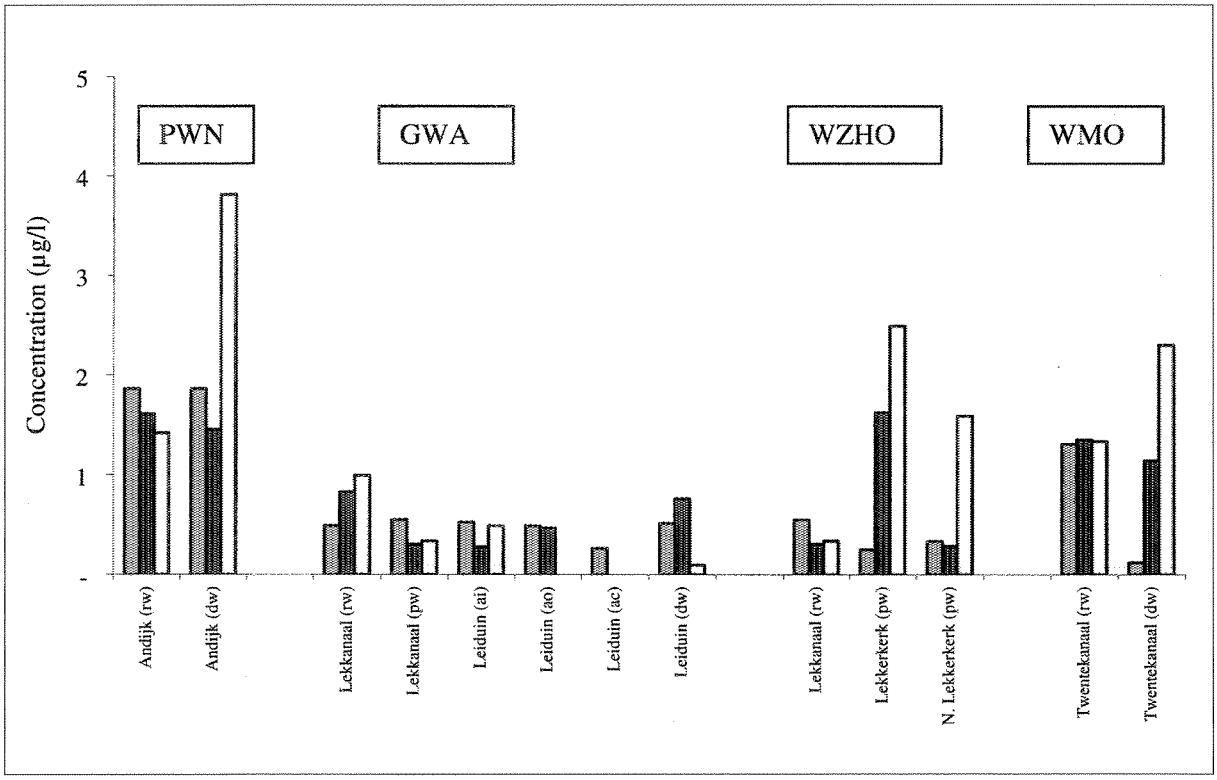

Fig. 7.10 The total phthalate load in raw water (rw), process water (pw) and in finished water (dw), prepared from Rhine water.

8. Conclusions and recommendations

The results of the present study confirm the occurrence of endocrine disrupting compounds in surface-, process-, and drinking water in the Meuse and Rhine basins.

Endocrine disrupting compounds were frequently detected in surface water, process water and in some individual cases at low levels in drinking water. Concentrations varied in some cases by more than four orders of magnitude, but the results of the ERC biotest did not exceed a value that would imply immediate oestrogenic effects on living organisms. Some samples of surface water, however, showed strikingly high concentrations.

With the limited number of analytes that were measured it was not possible to trace the origin of the observed oestrogenic effect. The results of the ERC assay could not be explained from the concentrations of the compounds analysed in this project. The response of the ERC assay was in some cases higher than could be explained on the basis of the individual response of the components. In another case, the total of the expected individual responses was higher than that of the ERC assay. Inhibiting and synergetic effects may have played a role in this respect.

The ADC/NOEC criterion, which is a measure for the toxicological risk produced by the individual compound, was frequently exceeded in surface water and higher than 0.01. In drinking water, however, the criterion was not exceeded.

Hormones are the most potent components as regards endocrine effects. Natural and synthetic hormones were only occasionally present in surface water, and were never observed in process water and drinking water. However, the detection limits are relatively high, and in some cases above the NOEC level.

The median concentration of bisphenol A in the Rhine is significantly higher than that in the Meuse, although a concentration of 21 µg/l was found at Brakel, a Meuse sampling site at the Afgedamde Maas in June.

Phthalates were generally found in many samples in surface, process and drinking water. The concentrations in the Meuse were significantly higher than in the Rhine, and the median concentration in September was significantly higher than in March or in June.

All products showed a significantly higher concentration in surface water than in process and drinking water. This indicates that the water production processes removed a large proportion of these pollutants.

The presence of oestrogens in drinking water was modest, and only low concentrations of phthalates were detected in the drinking water produced.

Alkylphenol polyethoxylates were only demonstrated in a few cases. It was remarkable that alkylphenols were not detected.

From the measured samples we can conclude that safe drinking water is produced. However, this study is unable to pronounce upon the production of safe drinking water in situations in which high concentrations of endocrinic disruptors are present in the extracted surface waters.

Recommendations

The finding of endocrine disruptors suggests the need for further study. The quantity of data provided by the present study is too low to give a complete and precise interpretation. It is obvious from the results that the concentration of the solutes can vary considerably. At high concentrations, there is no guarantee that the substances cannot break through the barriers of the water purification processes. It is therefore strongly recommended to intensify the number of water samples over a longer period, for instance, 10-12 samples over a whole year. This will give insight into the fluctuations in the concentrations of the various chemicals.

On the other hand, the number of sampling sites could be considerably reduced, for instance to two sites along the Rhine (Lobith and Lekkanaal) and two sites along the Meuse (Eijsden and Keizersveer). This would mean a considerable cost reduction.

Some of the analytes originally selected were not observed in this study. Before a new sampling and measurement programme is started, it would be wise to reconsider the selection of analytes. The spectrum of endocrine disrupting compounds may be changing and the choice of products to be measured should be restricted to the products that can be expected in the Meuse or Rhine.

The chemical analysis should be supported by biological tests. The ERC assay has proved to be of significant value for the demonstration of oestrogenic effects. The best test to demonstrate oestrogenic effects directly *in situ* is the vitellogenin test using male trout. Male trout have been shown to be sensitive to oestrogen exposure and are easy to keep under field conditions in flow-through cages. When exposed to oestrogen disruptors at sufficiently high concentration, male trout will produce the female egg-yolk protein (vitellogenin) in their blood plasma. The concentration is a measure of the exposed concentrations of oestrogenic contaminants to which the animals are exposed.

The results showed distinct variation in concentrations and occasionally very high levels of contaminants near drinking water company extraction points. It is not known if this may threaten safe drinking water production. Therefore study on theses compounds in surface water and process water needs further attention.

DZH's purification process, with its extraction point at Brakel, needs close monitoring since relatively high concentrations of contaminants can occur.

The industrial area near Köln generates contamination of surface waters by endocrine disruptors. The limited number of data show the presence of these substances and further studies should take place in surface water and drinking water.

The WZHO water supply company uses river bank infitration. The results demonstrated that river contaminations could only partly be removed. Therefore, further study of the drinking water is recommended.

Some of the Twentekanaal surface water samples contained oestrogenic substances. In one case the drinking water showed an increased response of the ERC assay. Further study into this matter is advisable.

The drinking water at the Andijk production plant is probably contaminated by phthalates during the process. The cause of this phenomenon should be studied in a new programme.

9. References

Adl 1999 H. Adlercreutz, *Phytoestrogens. State of the Art.* Environmental Toxicology and Pharmacology 7(1999)201-207.

Ahe 1993 M. Ahel, J. McEvoy and W. Giger, *Bioaccumulation of the lipophilic metabolites of non-ionic surfactants in freshwater organisms*, Environm. Pollut. 79(1993)243-248.

BBC 1993 Horizon: *Assault on the male*, London.

Bel 1999 A.C. Belfroid, A. van der Horst, A.D. Vethaak, A.J. Schäfer, G.B.J. Rijs, J. Wegener and W.P. Cofino, *Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands*, Sci. Total Envir., 225(1999)101-108.

Bel 2000 A.C. Belfroid, P. de Voogt, E. van der Velde and G. Rijs, *Speuren naar (xeno)oestrogene sporen*, , H2O, 33(2000)17-20.

Bla 1970 S.J.M. Blaber, *The occurrence of a penis like outgrowth behind the right tentacle in spent females of Nucella Lapillus (L.)*, Proc. Malacol. Soc. Lond., 39 (1970) 231 - 233.

Bos 1995 A.T.C. Bosveld, J. Gradener, A.J. Murk, A. Brouwer, M. van Kampen, E.H.G. Evers and M. van den Berg, *Effects of PCDD's, PCDF's and PCB's in common tern (Sterna hirundo) breeding in estuarine and coastal colonies in the Netherlands and Belgium*, Environmental Toxicology and Chemistry, 14 (1995) 99 – 115.

Boe 1995 M. Boelsma and B. Onrad, 'Onderzoek verband milieu en sperma', *Algemeen Dagblad*, 19 juli 1995.

Bro 1995 M. Villalobos, N. Olea, J.A. Brotons, M.F. Olea-Serrano, J. M. Ruiz de Almodovar, V. Pedraza, *The E-screen assay: A comparison of different MCF 7 cell stocks*,. Environmental health perspectives supplements, *103* (1995) 844-851.

Car 1993 E. Carlsen, A. Giwercman, N. Kieding and N.E. Skakkebaek, *Evidence for decreasing quality of semen during the past 50 years*, BMJ, 305 (1993) 609 – 613.

Col 1998 T. Colborn, *Building scientific consensus on endocrine disruptors*, Environ. Tox., 17(1998)1-2.

Den 1998 W.D. Denneman, N. Heeg, A.J. Palsma, H.M.J. Janssen, *Xeno-oestrogenen in drinkwater(bronnen)*, Kiwa, Nieuwegein.

Did 1995 R. Didde, 'Er lijkt toch wat loos met het sperma', *Volkskrant*, 16 september 1995

Dir 1995 S. Dirksen, T.J. Boudewijn, L.K. Slager, R.G. Mes, M.J.M. Van Schaick, and P. De Voogt, *Reduced breeding success of cormorants (Phalacrocorax carbo sinensis) in relation to persistent organochlorine pollution of aquatic habitats in the Netherlands*, Environ. Pollut. 88 (1995) 119-132.

Elliott 1987 J.E. Elliott, R.W. Butler, R.J. Norstrom and P.E. Whitehead, *Environmental contaminants and reproductive success of* Great Blue Herons *(Ardea herodias) in British Columbia*, Environm. Pollut., 59(1987)91-114.

Eur 1997 European Community. *European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife*. Workshop Publication EUR 17549 Paris, France.

Fox 1992 G.A. Fox in: T. Colborn and C. Clement (Editors), *Advances in modern environmental toxicology (XXI), Chemically-induced alterations in sexual and functional development: The Wildlife/Human connection*, Princeton, Princeton Scientific: 147-158.

Gez 1999 Gezondheidsraad, *Hormoonontregelaars in ecosystemen*, Den Haag.

Gre 1994 B. Greenstein, *Endocrinology at a Glance*, Blackwell Science Ltd, Oxford, 1994.

Gui 1994 L.J. Guillette, T.S. Gross, G.R. Masson, M.M. Matter, H.F. Parcival and A.R. Woodward, *Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida*, Environ. Health Perspect., 102 (1994) 680-688.

Har 1999 J.E. Harries, A. Janbakhsh, S. Jobling, P. Matthiesen, J.P. Sumpter and C.R. Tyler, *Estrogenic potency of effluent from two sewage treatment works in the United Kingdom*, Environ. Toxicol. Chem., 18(1999)932-937.

Hun 1977 G.L. Hunt and M.W. Hunt, *Female-female pairing in Western Gulls (Laris occidentalis) in southern California*, Science 196(1977)1466-1467.

Job 1993 S. Jobling and J.P. Sumpter, *Detergent components in sewage effluent are weakly oestrogenic to fish: An in vitro study using rainbow trout (Oncorhynchus mykiss) hepatocytes*, Aquat. Toxicol., 27(1993)361-372.

Job 1996 S. Jobling, D. Sheanan, J.A. Osborne, P. Matthiessen, J.P. Sumpter, *Inhibition of testicular growth in Rainbow Trout exposed to estrogenic alkylphenolic chemicals*, Environ. Toxicol. Chem., 15 (1996) 194-202.

Jun 1974 G.A. Junk, H.J. Svec, R.D. Vick and M.J. Avery, *Contamination of water by synthetic polymer tubes*, Environm. Sci. Technol., 8 (1974) 13.

Kas 1996 J. v.Kasteren, *Pseudo-oestrogenen in het milieu, beweringen gewogen*, Stichting C3, Amsterdam.

Leg 1999 J. Legler, C.E. van den Brink, A. Brouwer, A.J. Murk, P.T. van der Saag, A.D. Vethaak and B. van der Burg. *Development of a stably transfected estrogen receptor-mediated luciferase reporter gene assay in the human T47D breast cancer cell line*. Tox. Sciences, 48(1999)55-66.

Ler 1995 A. Lerchl, *Evidence for decreasing quality of sperm: Presentation of data on sperm was flawed*, BMJ, 311, no 7004(1995)569 – 570.

Mat 1998 P. Matthiessen, Y. Allen, C.R. Alchin e.a., *Oestrogenic endocrine disruption in flounder (Platichthys flesus) from United Kingdom estuarine and marine waters*, Sci. Ser. Tech. Rap. CEFAS Lowestoft 107(1998).

Men 1996 B.P. Mensink, C.C. ten Hallers-Tjabbes, J. Kralt, I.L. Freriks & J.P. Boon, *Assessment of imposex in the Common Whelk, Buccinum undatum (L.) from the Eastern Scheldt, The Netherlands*, Marine Environmental Research, 41 (1996) 315-325.

Mur 1999 Personal communication, december 1999.

Ols 1995 G.W. Olsen, C.E. Ross, K.M. Bodner, L.I. Lipschultz, and J.M. Ramlow, *Have sperm counts been reduced 50 percent in 50 years? A statistical model revisited*, Fertility and Sterility, *63* (1995) 887 – 893.

Pur 1994 C.E. Purdom, P.A. Hardiman and V.J. Bye, *Estrogenic effects of effluents from sewage treatment works*, Chem. Ecol., *8* (1994) 275-285.

Rei 1985 P.J.H. Reijnders, *Reproductive failure in common seals feeding on fish from polluted coastal waters*, , Nature, *324*, 456-457.

Rei 1997 P.J.H. Reijnders, E.H. Ries, S. Tougaard, N. Norgaard, G. Heidemann, J. Schwartz, E. Varechi and I.M. Traut, *Population development of harbour seals Phoca vitulina in the WaddenSea after the 1988 virus epizootic*, J.Sea Res., 38(1997)161-168. (Aanvraag Twente 4-2-00, f32)

Rou 1995 E.J. Routledge, J.P. Sumpter, *Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen*, Environ. Toxicol. Chem., *15* (1996) 241-248.

Rou 1998 E.J. Routledge, D.Sheanan, C. Desbrow, G.Brighty, M.Waldock, J.P. Sumpter, *Identification of oestrogenic chemicals in STW effluent: In vivo response in Trout and Roach*, Env. Sci. Technol., *32* (1998)1559-1565.

Rur 1977 R.D. Rurainski, H.J. Theiss and W. Zimmermann, *Über das Vorkommen von natürlichen und sythetische Oestrogenen im Trinkwasser*, Gwf-Wasser/Abwasser, 118-6(1977).

San 1994 Sanders, G.T., R.C. Ward, J.C. Loftis, T.D. Steele, D.D. Adrian & Vujica Yevjevich. *Design of networks for monitoring water quality.* Water resources publications. Highlands Ranch, USA 1994.

Sch 1970 R.W. Schreiber, *Breeding biology of Western Gulls (Larus occidentalis) on San Nicolas Island, California, 1968* , Condor 72 (1970) 133-140.

Sha 1998 R.M. Sharpe, *Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment: Environmental oestrogens and male fertility*, Pure & Appl. Chem., 70 (1998) 1685 - 1701.

Slo 1990 `W. Sloof, J.A. Janus, C.J.M. Koning, W.J.G.M. Peijnenburg, J.P.M. Ros, *Exploratory report phthalates*, RIVM-report no. 710401001, Bithoven 1990.

Sow 1980 A.L. Sowls, A.R. Degange, J.W. Nelson and G.S. Lester, *Catalog of California Seabird Colonies*, Coastal Ecosystems Project, Fish and Wildlife Service, U.S. Department of the Interior, Washington D.C. 1980, p.371.

Sta 2000 C.A.Staples, P.D. Dorn, G.M. Glecka, S.T. O'Block, D.R. Branson, L.R. Harris, *Bisphenol A concentrations in receiving waters near Us processing and manufacturing facilities*, Chemosphere, *40* (2000) 521-526.

Stu 1996 M. Stumpf, T.A. Ternes, K.Haberer and W.Baumann, *Nachweis von natürlichen und synthetischen Östrogenen in Kläranlagen und Fliessgewässern*, VomWasser, 87(1996)251-261.

Ter 1999 T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R-D. Wilken, M. Servos, *Behavior and occurrence of estrogens in municipal sewage plants-I. Investigations in Germany, Canada and Brazil*, Sci. Total Environ., 225 (1999) 81-90.

Tyl 1998 C.R. Tyler, E.J. Routledge. *Oestrogenic effects in fish in English rivers with evidence of their causation*, Pure and Applied Chemistry, *70(9)* (1998) 1793-1804.

Vel 2000 E. van der Velde, *Method for the analysis of phthalates in environmental samples,* personal communication with the author, RIVM Bithoven, february 2000.

Voo 2000 P. Voogd, *Method for the analysis of alkylphenol ethoxylates in water samples,* personal communication with the author, MTC, Amsterdam, february 2000.

Wag 1999 A.J. Wagenvoort , *Oestrogeen-active stoffen in zoetwatersystemen*, WBB-rapport, Werkendam.

Weg 1999 G.Wegener, J.Persin, F. Karrenbrock, O. Rörden and I. Hübner, *Vorkommen und verhalten von natürlichen und synthetischen Östrogenen und deren Konjugaten in der aquatischen Umwelt*, Vom Wasser, 92(1999)347-360.

Appendix 1

Tables of the analytical results of the water samples

The tables are arranged per analytical parameter, i.e. ER-CALUX assay, hormones and bifsphenol A, phthalates, the alkylphenoxy ethoxylates and the general parameters chloride, salinity, suspended particles and dry residue. Within each parameter the results are arranged per phase and per sampling site.

Phase 1 March 1999 ER-CALUX assay Meuse

Location	Company	Description	Sample code	Sample date	EEQ (pMol/l)	EEQ (ng/l)
Surface water						
Remilly	BIWM	French-Belgian border	W.OW.1.REM.YES.F	8-3-99	0,049	0,013
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.1.TAI.YES.F	8-3-99	0,039	0,011
Namêche	AWW	Meuse	W.OW.1.NAM.YES.F	8-3-99	0,108	0,029
Liège	AWW	Albert kanaal	W.OW.1.LUI.YES.F	8-3-99	0,073	0,020
Eijsden	RIZA	Belgian-Dutch border	A.OW.1.EYS.YES.F	23-3-99	0,565	0,154
Belfeld	RW-DL	Meuse (NL)	W.OW.1.BEL.YES.F	9-3-99	0,045	0,012
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.1.KEI.YES.F	9-3-99	0,242	0,066
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.1.WBB.YES.F	9-3-99	0,103	0,028
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.1.BRA.YES.F	8-3-99	0,124	0,034
					minimum	0,011
					maximum	0,154
					mediaan	0,028
					90 percentiel	0,083
Processwater						
Petrusplaat	WBB	Finished product	W.LW.1.WBB.ERC.O	9-3-99	0,103	0,028
Scheveningen	DZH	Collected after extraction	W.LW.1.DZ2.ERC.O	8-3-99	< 0,019	<* 0,005
Drinking water						
Scheveningen	DZH	Collected after filtration	W.LW.1.DZ1.ERC.O	8-3-99	< 0,014	<* 0,004
					minimum	0,004
					maximum	0,028

Legend: EEQ = oestradiol equivalents; <* = results are an indication for they are beyond the levels of certain quantification

Phase 1 **March 1999** **ER-CALUX assay Rhine**

Location	Company	Description	Sample code	Sample date	EEQ (pMol/l)	EEQ (ng/l)
Surface water						
Karlsruhe	TZW	Rhine (D)	W.OW.1.KRL.YES.F	8-3-99	0,091	0,025
Köln	GEW	Site 1	W.OW.1.KO1.YES.F	8-3-99	0,043	0,012
Köln	GEW	Site 2	W.OW.1.KO2.YES.F	8-3-99	0,045	0,012
Köln	GEW	Site 3	W.OW.1.KO3.YES.F	8-3-99	0,083	0,023
Köln	GEW	Site 4	W.OW.1.KO4.YES.F	8-3-99	< 0,024	<* 0,007
Köln	GEW	Site 5	W.OW.1.KO5.YES.F	8-3-99	0,035	0,010
Köln	GEW	Site 6	W.OW.1.KO6.YES.F	8-3-99	0,135	0,037
Lobith	RIZA	German-Dutch border	A.OW.1.LOB.YES.F	15-3-99	0,108	0,029
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.1.LKN.YES.F	8-3-99	0,043	0,012
Andijk	PWN	Inlet at IJsselmeer	W.OW.1.AND.YES.F	8-3-99	0,036	0,010
Twentekanaal	WMO	Inlet WMO	W.OW.1.WMO.YES.F	8-3-99	0,098	0,027
					minimum	0,007
					maximum	0,037
					mediaan	0,012
					90 percentiel	0,029
Processwater						
Nieuwegein	WRK	Finished product	W.LW.1.LKN.ERC.O	8-3-99	< 0,016	<* 0,004
Lekkerkerk	WZHO	GLS PF 99	W.OW.1.WZ1.YES.F	8-3-99	0,052	0,014
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.1.WZ2.YES.F	8-3-99	0,037	0,010
Leiduin	GWA	After infitration (a.i.)	W.LW.1.GW2.ERC.O	8-3-99	0,043	0,012
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.1.GW3.ERC.O	8-3-99	< 0,019	<* 0,005
Leiduin	GWA	After active carbon filtration (a.c.)	W.LW.1.GW4.ERC.O	8-3-99	< 0,012	<* 0,003
Drinkingwater						
Leiduin	GWA	Drinking water	W.LW.1.GW1.ERC.O	8-3-99	< 0,018	<* 0,005
Andijk	PWN	Drinking water	W.LW.1.AND.ERC.O	8-3-99	0,03	0,008
Twentekanaal	WMO	Drinking water	W.LW.1.WMO.ERC.O	8-3-99	< 0,014	<* 0,004
					minimum	0,003
					maximum	0,014

Legend: EEQ = oestradiol equivalents; <* = results are an indication for they are beyond the levels of certain quantification

Phase 2 **June 1999** **ER-CALUX assay Meuse**

Location	Company	Description	Sample code	Sample date	EEQ (pMol/l)	EEQ (ng/l)
Maas						
Remilly	BIWM	French-Belgian border	W.OW.2.REM.YES.F	6/28/99	<* 0,00067	< 0,00018
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.2.TAI.YES.F	6/28/99	0,027	0,007
Namêche	AWW	Meuse	W.OW.2.NAM.YES.F	6/28/99	0,003	0,001
Liège	AWW	Albert kanaal	W.OW.2.LUI.YES.F	6/28/99	0,011	0,003
Eijsden	RIZA	Belgian-Dutch border	A.OW.2.EYS.YES.F	7/13/99	0,080	0,022
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.2.WPH.YES.F	6/30/99	0,115	0,031
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.2.WPR.YES.F	6/30/99	0,069	0,019
Belfeld	RW-DL	Meuse (NL)	W.OW.2.BEL.YES.F	6/29/99	0,035	0,009
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.2.KEI.YES.F	6/29/99	0,048	0,013
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.2.WBB.YES.F	6/28/99	0,047	0,013
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.2.BRA.YES.F	6/28/99	0,017	0,005
					minimum	0,0002
					maximum	0,0313
					mediaan	0,0095
					90 percentiel	0,0217
Process water						
Petrusplaat	WBB	Finished product	W.LW.2.LKN.ERC.O	6/28/99	<* 0,00101	< 0,00028
Scheveningen	DZH	Collected after extraction	W.LW.2.DZ1.ERC.O	6/28/99	<* 0,006	< 0,002
Ouddorp	Delta	Collected process water	W.OW.2.OUD.YES.F	6/29/99	<* 0,001	< 0,0002
Drinking water						
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.2.DZ2.ERC.O	6/28/99	<* 0,004	< 0,001
Ouddorp	Delta	Drinking water	W.LW.2.OUD.ERC.O	6/29/99	0,077	0,021
Braakman	Delta	Drinking water	W.LW.2.BRA.ERC.O	6/29/99	0,036	0,010
Kralingen	WBE	Drinking water	W.LW.2.WB1.ERC.O	6/30/99	0,027	0,007
Berenplaat	WBE	Drinking water	W.LW.2.WB2.ERC.O	6/30/99	0,082	0,022
					minimum	0,000
					maximum	0,022

Legend: EEQ = oestradiol equivalents; <* = results are an indication for they are beyond the levels of certain quantification

Phase 2 **June 1999** **ER-CALUX assay Rhine**

Location	Company	Description	Sample code	Sample date	EEQ (pMol/l)	EEQ (ng/l)
Surface water						
Lobith	RIZA	German-Dutch border	A.OW.2.LOB.YES.F	05-07-99	0,038	0,010
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.2.LKN.YES.F	6/30/99	0,052	0,014
Andijk	PWN	Inlet at IJsselmeer	W.OW.2.AND.YES.F	6/28/99	0,143	0,039
Twentekanaal	WMO	Inlet WMO	W.OW.2.WMO.YES.F	6/28/99	0,066	0,018
					minimum	0,010
					maximum	0,039
					mediaan	0,016
					90 percentiel	0,033
Process water						
Nieuwegein	WRK	Finished product	W.LW.2.LKN.ERC.O	6/30/99	0,015	0,004
Lekkerkerk	WZHO	GLS PF 99	W.OW.2.WZ1.YES.F	6/28/99	0,035	0,009
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.2.WZ2.YES.F	6/28/99	<* 0,001	< 0,0002
Leiduin	GWA	After infitration (a.i.)	W.LW.2.GW2.ERC.O	6/29/99	<* 0,003	< 0,001
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.2.GW3.ERC.O	6/29/99	<* 0,003	< 0,001
Drinking water						
Leiduin	GWA	Drinking water	W.LW.2.GW1.ERC.O	6/29/99	0,002	0,00041
Weesperkarspel	GWA	Drinking water	W.LW.2.GW4.ERC.O	6/29/99	<* 0,001	< 0,000
Andijk	PWN	Drinking water	W.LW.2.AND.ERC.O	6/28/99	0,065	0,018
Twentekanaal	WMO	Drinking water	W.OW.2.WMO.YES.F	6/28/99	0,739	0,201
					minimum	0,000
					maximum	0,201

Legend: EEQ = oestradiol equivalents; <* = results are an indication for they are beyond the levels of certain quantification

Fase 3 **September 1999** **ER-CALUX assay Meuse**

Location	Company	Description	Sample code	Sample date	EEQ (pMol/l)	EEQ (ng/l)
Surface water						
Remilly	BIWM	French-Belgian border	W.OW.3.REM.YES.F		0,161	0,044
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.3.TAI.YES.F		0,094	0,026
Namêche	AWW	Meuse	W.OW.3.NAM.YES.F		0,053	0,014
Liège	AWW	Albert kanaal	W.OW.3.LUI.YES.F		0,058	0,016
Eijsden	RIZA	Belgian-Dutch border				
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.3.WPH.YES.F		0,073	0,020
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.3.WPR.YES.F		0,077	0,021
Belfeld	RW-DL	Meuse (NL)	W.OW.3.BEL.YES.F		0,558	0,152
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.3.KEI.YES.F		0,611	0,166
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.3.WBB.YES.F		0,081	0,022
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.3.BRA.YES.F		0,093	0,025
					minimum	0,014
					maximum	0,166
					mediaan	0,024
					90 percentiel	0,153
Petrusplaat	WBB	Finished product	W.LW.3.WBB.ERC.O		0,021	0,006
Scheveningen	DZH	Collected after extraction	W.LW.3.DZ2.ERC.O		0,071	0,019
Ouddorp	Delta	Collected process water	W.OW.3.OUD.YES.F		0,05	0,014
Drinking water						
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.3.DZ1.ERC.O		<	<
Ouddorp	Delta	Drinking water	W.LW.3.OUD.ERC.O		<	<
Braakman	Delta	Drinking water	W.LW.3.BRA.ERC.O		<	<
Kralingen	WBE	Drinking water	W.LW.3.WB1.ERC.O		<	<
Berenplaat	WBE	Drinking water	W.LW.3.WB2.ERC.O		<	<
					minimum	0,006
					maximum	0,019

Fase 3 — September 1999 — ER-CALUX assay Rhine

Location	Company	Description	Sample code	Sample date	EEQ (pMol/l)	EEQ (ng/l)
Surface water						
Lobith	RIZA	German-Dutch border	A.OW.3.LOB.HOR.F			
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.3.LKN.YES.F		0,166	0,045
A'dam-Rijnkanaal	GWA	Inlet Weesperkarspel	W.OW.3.GW1.ERC.F		0,035	0,010
Andijk	PWN	Inlet at IJsselmeer	W.OW.3.AND.YES.F		0,132	0,036
Twentekanaal	WMO	Inlet WMO	W.OW.3.WMO.YES.F		0,041	0,011
					minimum	0,010
					maximum	0,045
					mediaan	0,024
					90 percentiel	0,042
Process water						
Nieuwegein	WRK	Finished product	W.LW.3.LKN.ERC.O		<	
Lekkerkerk	WZHO	GLS PF 99	W.OW.3.WZ1.YES.F		0,036	0,010
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.3.WZ2.YES.F		0,145	0,039
Leiduin	GWA	After infitration (a.i.)	W.LW.3.GW2.ERC.O		<	
Drinking water						
Leiduin	GWA	Drinking water	W.LW.3.GW1.ERC.O		<	
Weesperkarspel	GWA	Drinking water	W.LW.3.GW3.ERC.O		<	
Andijk	PWN	Drinking water	W.LW.3.AND.ERC.O		0,017	0,005
Twentekanaal	WMO	Drinking water	W.LW.3.WMO.ERC.O		<	
					minimum	0,005
					maximum	0,039

Phase 1 **March 1999** **Hormones and Bisphenol A analysis Meuse**

Location	Company	Description	Sample code	Sample date	17α-oestradiol	17β-oestradiol	oestron	17α-ethinyloestradiol	bisphenol A
Surface water					Concentrations in ng/l				
Remilly	BIWM	French-Belgian border	W.OW.1.REM.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,9
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.1.TAI.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,8
Namêche	AWW	Meuse	W.OW.1.NAM.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,8
Liège	AWW	Albert kanaal	W.OW.1.LUI.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	66
Eijsden	RIZA	Belgian-Dutch border	A.OW.1.EYS.HOR.F	23-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 18,1
Belfeld	RW-DL	Meuse (NL)	W.OW.1.BEL.HOR.F	9-3-99	< 0,3	< 0,8	< 0,3	< 0,3	42
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.1.KEI.HOR.F	9-3-99	< 0,3	< 0,8	< 0,7	< 0,3	38
WBB	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.1.WBB.HOR.F	9-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 26
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.1.BRA.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,8
				minimum	0,3	0,8	0,3	0,3	8,8
				maximum	0,3	0,8	0,7	0,3	66
				mediaan	0,3	0,8	0,3	0,3	18,1
				90 percentiel	0,3	0,8	0,38	0,3	46,8
Processwater									
Petrusplaat	WBB	Finiahed product	W.LW.1.WBB.HOR.O	9-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 9
Scheveningen	DZH	Collected after extraction	W.LW.1.DZ2.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,9
Drinking water									
Scheveningen	DZH	Collected after filtration	W.LW.1.DZ1.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,9
				minimum	0,3	0,8	0,3	0,3	8,9
				maximum	0,3	0,8	0,3	0,3	9

Legend: <* = results are an indication for they are beyond the levels of certain quantification

Phase 1 **March 1999** **Hormones and Bisphenol A analysis Rhine**

Location	Company	Description	Sample code	Sample date	17α-oestradiol	17β-oestradiol	oestron	17α-ethinyloestradiol	bisphenol A
Surface water					Concentrations in ng/l				
Karlsruhe	TZW	Rhine (D)	W.OW.1.KRL.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,8
Köln	GEW	Site 1	W.OW.1.KO1.HOR.F	8-3-99	< 0,3	< 0,8	< 0,7	< 0,3	240
Köln	GEW	Site 2	W.OW.1.KO2.HOR.F	8-3-99	< 0,3	< 0,8	2,2	< 0,3	190
Köln	GEW	Site 3	W.OW.1.KO3.HOR.F	8-3-99	< 0,3	< 1	< 0,3	< 0,3	210
Köln	GEW	Site 4	W.OW.1.KO4.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	200
Köln	GEW	Site 5	W.OW.1.KO5.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	1000
Köln	GEW	Site 6	W.OW.1.KO6.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	93
Lobith	RIZA	German-Dutch border	A.OW.1.LOB.HOR.F	15-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 18,2
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.1.LKN.HOR.F	8-3-99	< 0,3	< 0,8	< 0,6	< 0,3	< 29
Andijk	PWN	Inlet at IJsselmeer	W.OW.1.AND.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,8
Twentekanaal	WMO	Inlet WMO	W.OW.1.WMO.HOR.F	8-3-99	< 0,3	< 0,8	1,6	< 0,3	39
				minimum	0,3	0,8	0,3	0,3	8,8
				maximum	0,3	1	2,2	0,3	1000
				mediaan	0,3	0,8	0,3	0,3	93
				90 percentiel	0,3	0,8	1,6	0,3	240
Proceswater									
Lekkanaal	WRK	Finished product	W.LW.1.LKN.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 9,2
Lekkerkerk	WZHO	GLS PF 99A	W.OW.1.WZ1.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	130
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.1.WZ2.HOR.F	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	99
Leiduin	GWA	After infitration (a.i.)	W.LW.1.GW2.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,9
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.1.GW3.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,8
Leiduin	GWA	After active carbon filtration (a.c.)	W.LW.1.GW4.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,9
Drinkwater									
Leiduin	GWA	Drinking water	W.LW.1.GW1.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,9
Andijk	PWN	Drinking water	W.LW.1.AND.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,9
Twentekanaal	WMO	Drinking water	W.LW.1.WMO.HOR.O	8-3-99	< 0,3	< 0,8	< 0,3	< 0,3	< 8,8
				minimum	0,3	0,8	0,3	0,3	8,8
				maximum	0,3	0,8	0,3	0,3	130

Legend: <* = results are an indication for they are beyond levels of certain quantification

Phase 2 **June 1999** **Hormones and Bisphenol A analysis Meuse**

Location	Company	Description	Sample code	Sample date	17α-oestradiol	17β-oestradiol	oestron	17α-ethinyl-oestradiol	bisphenol A
Surface water					Concentrations in ng/l				
Remilly	BIWM	French-Belgian border	W.OW.2.REM.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,8
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.2.TAI.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
Namêche	AWW	Meuse	W.OW.2.NAM.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 23
Liège	AWW	Albert kanaal	W.OW.2.LUI.HOR.F	28-6-99	< 0,3	< 0,8	4	< 0,3	490
Eijsden	RIZA	Belgian-Dutch border	W.OW.2.EYS.HOR.F	13-7-99	< 0,4	< 0,8	1,3	< 0,3	< 11
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.2.WPH.HOR.F	30-6-99	< 0,3	< 0,8	< 0,7	< 0,3	45
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.2.WPR.HOR.F	30-6-99	< 0,3	< 0,8	2,1	< 0,3	100
Belfeld	RW-DL	Meuse (NL)	W.OW.2.BEL.HOR.F	29-6-99	< 0,3	< 0,8	1,1	< 0,3	39
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.2.KEI.HOR.F	29-6-99	< 0,3	< 0,8	< 0,4	< 0,3	230
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.2.WBB.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 27
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.2.BRA.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	21220
				minimum	0,3	0,8	0,3	0,3	10,8
				maximum	0,4	0,8	4	0,3	21220
				mediaan	0,3	0,8	0,4	0,3	39
				90 percentiel	0,3	0,8	2,1	0,3	490
Proceswater									
Petrusplaat	WBB	Finished product	W.LW.2.WBB.HOR.O	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
Scheveningen	DZH	Collected after extraction	W.LW.2.DZ2.HOR.O	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,7
Ouddorp	Delta	Collected process water	W.OW.2.OUD.HOR.F	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
Drinking water									
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.2.DZ1.HOR.O	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,7
Ouddorp	Delta	Drinking water	W.LW.2.OUD.HOR.O	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11
Braakman	Delta	Drinking water	W.LW.2.BRA.HOR.O	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,1
Kralingen	WBE	Drinking water	W.LW.2.WB1.HOR.O	30-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11
Berenplaat	WBE	Drinking water	W.LW.2.WB2.HOR.O	30-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11
				minimum	0,3	0,8	0,3	0,3	10,7
Legend:<* = results are an indication for they are beyond levels of certain quantification				maximum	0,3	0,8	0,3	0,3	11,1

Phase 2 June 1999 Hormones and Bisphenol A analysis Rhine

Location	Company	Description	Sample code	Sample date	17α-oestradiol	17β-oestradiol	oestron	17α-ethinyl-oestradiol	bisphenol A
					Concentrations in ng/l				
Surface water									
Lobith	RIZA	German-Dutch border	A.OW.2.LOB.HOR.F	5-7-99	< 0,3	< 0,8	< 0,3	< 0,3	43
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.2.LKN.HOR.F	30-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 35
Andijk	PWN	Inlet at IJsselmeer	W.OW.2.AND.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,8
Twentekanaal	WMO	Inlet WMO	W.OW.2.WZ1.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	150
				minimum	0,3	0,8	0,3	0,3	10,8
				maximum	0,3	0,8	0,3	0,3	150
				mediaan	0,3	0,8	0,3	0,3	39
				90 percentiel	0,3	0,8	0,3	0,3	117,9
Process water									
Nieuwegein	WRK	Finished product	W.LW.2.LKN.HOR.O	30-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
Lekkerkerk	WZHO	GLS PF 99	W.OW.2.WZ2.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	89
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.2.WMO.HOR.F	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 30
Leiduin	GWA	After infitration (a.i.)	W.LW.2.GW2.HOR.O	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,8
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.2.GW3.HOR.O	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
Drinking water									
Leiduin	GWA	Drinking water	W.LW.2.GW1.HOR.O	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
Weesperkarspel	GWA	Drinking water	W.LW.2.GW4.HOR.O	29-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,8
Andijk	PWN	Drinking water	W.LW.2.AND.HOR.O	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
Twentekanaal	WMO	Drinking water	W.LW.2.WMO.HOR.O	28-6-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
				minimum	0,3	0,8	0,3	0,3	10,8
				maximum	0,3	0,8	0,3	0,3	89

Legend: <* = results are an indication for they are beyond the levels of certain quantification

Phase 3 September 1999 Hormones and Bisphenol A analysis Meuse

Location	Company	Description	Sample code	Sample date	17α-oestradiol	17β-oestradiol	oestron	17α-ethinyl-oestradiol	bisphenol A
					Concentrations in ng/l				
Surface water									
Remilly	BIWM	French-Belgian border	W.OW.3.REM.HOR.F	20-9-99	< 0,3	< 0,8	<* 0,7	< 0,3	< 11,1
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.3.TAI.HOR.F	20-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,3
Namêche	AWW	Meuse	W.OW.3.NAM.HOR.F	20-9-99	< 0,3	< 0,8	1,3	< 0,3	71
Liège	AWW	Albert kanaal	W.OW.3.LUI.HOR.F	20-9-99	< 0,3	< 0,9	1,2	< 0,3	580
Eijsden	RIZA	Belgian-Dutch border	A.OW.3.EYS.HOR.F	-					
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.3.WPH.HOR.F	21-9-99	< 0,3	< 0,8	<* 0,9	< 0,3	< 19
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.3.WPR.HOR.F	21-9-99	< 0,3	< 0,8	1,1	< 0,3	82
Belfeld	RW-DL	Meuse (NL)	W.OW.3.BEL.HOR.F	21-9-99	< 0,3	< 0,8	1,5	< 0,3	< 17
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.3.KEI.HOR.F	21-9-99					
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.3.WBB.HOR.F	20-9-99	< 0,3	< 0,8	1,2	< 0,3	< 11,3
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.3.BRA.HOR.F	20-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,2
				minimum	0,3	0,8	0,3	0,3	11,1
				maximum	0,3	0,9	1,5	0,3	580
				mediaan	0,3	0,8	1,1	0,3	17
				90 percentiel	0,3	0,82	1,34	0,3	181,6
Process water									
Petrusplaat	WBB	Finished product	W.LW.3.WBB.HOR.O	20-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,4
Scheveningen	DZH	Collected after extraction	W.LW.3.DZ2.HOR.O	21-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,2
Ouddorp	Delta	Collected process water	W.OW.3.OUD.HOR.F	21-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,2
Drinking water									
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.3.DZ1.HOR.O	21-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,1
Ouddorp	Delta	Drinking water	W.LW.3.OUD.HOR.O	21-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,6
Braakman	Delta	Drinking water	W.LW.3.BRA.HOR.O	21-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,5
Kralingen	WBE	Drinking water	W.LW.3.WB1.HOR.O	22-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 10,9
Berenplaat	WBE	Drinking water	W.LW.3.WB2.HOR.O	22-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11
				minimum	0,3	0,8	0,3	0,3	10,9
				maximum	0,3	0,8	0,3	0,3	11,6

Legend: <* = results are an indication for they are beyond the levels of certain quantification

Phase 3 September 1999 — Hormones and Bisphenol A analysis Rhine

Location	Company	Description	Sample code	Sample date	17α-oestradiol	17β-oestradiol	oestron	17α-ethinyl-oestradiol	bisphenol A
					Concentrations in ng/l				
Surface water									
Lobith	RIZA	German-Dutch border	A.OW.3.LOB.HOR.F	-					
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.3.LKN.HOR.F	22-9-99	< 0,3	< 0,8	<* 0,7	< 0,3	< 17
A'dam-Rijnkan	GWA	Inlet Weesperkarspel	W.OW.3.GW1.HOR.F	22-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 13
Andijk	PWN	Inlet at IJsselmeer	W.OW.3.AND.HOR.F	20-9-99	< 0,3	< 0,9	< 0,3	< 0,3	< 12,1
Twentekanaal	WMO	Inlet WMO	W.OW.3.WMO.HOR.F	20-9-99	< 0,3	< 0,8	<* 0,9	< 0,3	< 16
				minimum	0,3	0,8	0,3	0,3	12,1
				maximum	0,3	0,9	0,9	0,3	17
				mediaan	0,3	0,8	0,5	0,3	14,5
				90 percentiel	0,3	0,87	0,84	0,3	16,7
Process water									
Nieuwegein	WRK	Finished product	W.LW.3.LKN.HOR.O	22-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,1
Lekkerkerk	WZHO	GLS PF 99	W.OW.3.WZ1.HOR.F	20-9-99	< 0,3	< 0,8	< 0,3	< 0,3	100
Nieuw Lekkerla	WZHO	GPU PE 99B (De Put)	W.OW.3.WZ2.HOR.F	20-9-99	< 0,3	< 0,8	< 0,3	< 0,3	83
Leiduin	GWA	After infitration (a.i.)	W.LW.3.GW2.HOR.O	22-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11
Drinking water									
Leiduin	GWA	Drinking water	W.LW.3.GW1.HOR.O	22-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11
Weesperkarspel	GWA	Drinking water	W.LW.3.GW3.HOR.O	22-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11
Andijk	PWN	Drinking water	W.LW.3.AND.HOR.O	20-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,3
Twentekanaal	WMO	Drinking water	W.LW.3.WMO.HOR.O	20-9-99	< 0,3	< 0,8	< 0,3	< 0,3	< 11,4
				minimum	0,3	0,8	0,3	0,3	11
				maximum	0,3	0,8	0,3	0,3	100

Legend: <* = results are an indication for they are beyond the levels of certain quantification

Phase 1 **March 1999** **Phthalates analysis Meuse**

Location	Company	Description	Sample code	Sample date	DMP	DEP	DPP	DMPP	DBP
					Concentrations in µg/l				
Surface water									
Remilly	BIWM	French-Belgian border	W.OW.1.REM.FTA.F	8-3-99	< 0,002	< 0,003	< -0,026	0,250	0,127
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.1.TAI.FTA.F	8-3-99	< 0,003	< 0,022	< -0,021	0,237	0,102
Namêche	AWW	Meuse	W.OW.1.NAM.FTA.F	8-3-99	0,008	0,089	< -0,026	0,260	0,151
Liège	AWW	Albert kanaal	W.OW.1.LUI.FTA.F	8-3-99	0,016	0,245	< 0,022	0,379	0,276
Eijsden	RIZA	Belgian-Dutch border	A.OW.1.EYS.FTA.F	23-3-99	< 0,005	< 0,116	< -0,002	0,297	0,314
Belfeld	RW-DL	Meuse (NL)	W.OW.1.BEL.FTA.F	9-3-99	0,022	< 0,140	< -0,025	0,646	0,461
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.1.KEI.FTA.F	9-3-99	0,008	< 0,024	< 0,002	0,404	0,108
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.1.WBB.FTA.F	9-3-99	0,019	< 0,047	< -0,023	0,526	0,161
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.1.BRA.FTA.F	8-3-99	< 0,006	< -0,016	< -0,001	0,383	0,203
				minimum	0,002	-0,016	-0,026	0,237	0,102
				maximum	0,022	0,245	0,022	0,646	0,461
				mediaan	0,008	0,047	-0,021	0,379	0,161
				90 percentiel	0,020	0,161	0,006	0,550	0,343
Processwater									
Petrusplaat	WBB	Finished product	W.LW.1.WBB.FTA.O	9-3-99	< 0,003	< 0,019	< -0,024	0,576	0,321
Scheveningen	DZH	Collected after extraction	W.LW.1.DZ2.FTA.O	8-3-99	< 0,004	< 0,057	< -0,023	0,127	0,127
Drinking water									
Scheveningen	DZH	Collected after filtration	W.LW.1.DZ1.FTA.O	8-3-99	< 0,001	< 0,037	< -0,025	0,197	0,092
				minimum	0,001	0,019	-0,025	0,127	0,092
				maximum	0,004	0,057	-0,023	0,576	0,321

Phase 1 **March 1999** **Phthalates analysis Meuse**

continued

Location	Company	Description	Sample code	Sample date	BBP	DCHP	DEHP	DOP
Surface water					Concentrations in µg/l			
Remilly	BIWM	French-Belgian border	W.OW.1.REM.FTA.F	8-3-99	0,030	< 0,000	0,207	< 0,002
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.1.TAI.FTA.F	8-3-99	0,022	< 0,000	0,218	< 0,004
Namêche	AWW	Meuse	W.OW.1.NAM.FTA.F	8-3-99	0,072	< 0,000	0,297	< 0,003
Liège	AWW	Albert kanaal	W.OW.1.LUI.FTA.F	8-3-99	0,140	< 0,001	0,284	< 0,006
Eijsden	RIZA	Belgian-Dutch border	A.OW.1.EYS.FTA.F	23-3-99	0,092	< 0,002	0,283	< 0,004
Belfeld	RW-DL	Meuse (NL)	W.OW.1.BEL.FTA.F	9-3-99	0,242	< 0,002	0,185	< 0,006
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.1.KEI.FTA.F	9-3-99	0,035	< 0,003	0,405	< 0,006
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.1.WBB.FTA.F	9-3-99	0,045	< 0,002	0,185	< 0,005
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.1.BRA.FTA.F	8-3-99	0,093	< 0,001	0,181	0,014
				minimum	0,022	0,000	0,181	0,002
				maximum	0,242	0,003	0,405	0,014
				mediaan	0,072	0,001	0,218	0,005
				90 percentiel	0,160	0,002	0,319	0,008
Processwater								
Petrusplaat	WBB	Finished product	W.LW.1.WBB.FTA.O	9-3-99	< 0,010	< 0,001	< 0,099	< 0,004
Scheveningen	DZH	Collected after extraction	W.LW.1.DZ2.FTA.O	8-3-99	< 0,017	< 0,002	0,239	< 0,003
Drinking water								
Scheveningen	DZH	Collected after filtration	W.LW.1.DZ1.FTA.O	8-3-99	< 0,014	0,000	< 0,128	< 0,004
				minimum	0,010	0,000	0,099	0,003
				maximum	0,017	0,002	0,239	0,004

Phase 1 March 1999 Phthalates analysis Rhine

Location	Company	Description	Sample code	Sample date	DMP	DEP	DPP	DMPP	DBP
Surface water					Concentrations in µg/l				
Karlsruhe	TZW	Rhine (D)	W.OW.1.KRL.HOR.F	8-3-99	0,024	< 0,019	< 0,011	0,515	0,149
Köln	GEW	Site 1	W.OW.1.KO1.HOR.F	8-3-99	0,014	< -0,042	< 0,001	0,262	< 0,101
Köln	GEW	Site 2	W.OW.1.KO2.HOR.F	8-3-99	0,020	< -0,016	< 0,001	0,346	0,158
Köln	GEW	Site 3	W.OW.1.KO3.HOR.F	8-3-99	0,020	< 0,045	< 0,001	0,239	0,224
Köln	GEW	Site 4	W.OW.1.KO4.HOR.F	8-3-99	0,023	< 0,001	< 0,011	0,271	0,246
Köln	GEW	Site 5	W.OW.1.KO5.HOR.F	8-3-99	0,017	< 0,026	< 0,004	0,221	0,235
Köln	GEW	Site 6	W.OW.1.KO6.HOR.F	8-3-99	0,009	< 0,032	< 0,000	0,308	0,208
Lobith	RIZA	German-Dutch border	A.OW.1.LOB.HOR.F	15-3-99	0,010	< 0,052	< -0,004	0,128	< 0,060
Lekkanaal	WRK	Lekkanaal: inlet	W.OW.1.LKN.HOR.F	8-3-99	0,014	< -0,045	< 0,001	0,140	< 0,105
Andijk	PWN	Inlet at IJsselmeer	W.OW.1.AND.HOR.F	8-3-99	0,013	< 0,007	< 0,000	0,428	0,496
Twentekanaal	WMO	Inlet WMO	W.OW.1.WMO.HOR.F	8-3-99	0,036	< 0,002	< 0,000	0,493	0,244
				minimum	0,009	-0,045	-0,004	0,128	0,060
				maximum	0,036	0,052	0,011	0,515	0,496
				mediaan	0,017	0,007	0,001	0,271	0,208
				90 percentiel	0,024	0,045	0,011	0,493	0,246
Proceswater									
Lekkanaal	WRK	Finished product	W.LW.1.LKN.HOR.O	8-3-99	< 0,006	< 0,033	< 0,001	0,208	< 0,076
Lekkerkerk	WZHO	GLS PF 99A	W.OW.1.WZ1.HOR.F	8-3-99	< 0,002	< -0,007	< 0,000	0,095	< 0,085
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.1.WZ2.HOR.F	8-3-99	0,008	< -0,024	< -0,001	0,121	0,136
Leiduin	GWA	After infitration (a.i.)	W.LW.1.GW2.HOR.O	8-3-99	< 0,001	< -0,032	< -0,001	0,332	0,144
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.1.GW3.HOR.O	8-3-99	< 0,001	< -0,042	< -0,001	0,325	0,155
Leiduin	GWA	After active carbon filtration (a.c.)	W.LW.1.GW4.HOR.O	8-3-99	< 0,000	< -0,055	< 0,001	0,190	< 0,033
Drinkwater									
Leiduin	GWA	Drinking water	W.LW.1.GW1.HOR.O	8-3-99	< 0,000	< -0,079	< 0,005	< 0,225	0,090
Andijk	PWN	Drinking water	W.LW.1.AND.HOR.O	8-3-99	0,013	< 0,007	< 0,000	0,428	0,496
Twentekanaal	WMO	Drinking water	W.LW.1.WMO.HOR.O	8-3-99	0,000	< -0,078	< -0,001	< 0,077	< 0,005
				minimum	0,000	-0,079	-0,001	0,077	0,005
				maximum	0,013	0,033	0,005	0,428	0,496

Phase 1 **March 1999** **Phthalates analysis Rhine**

continued

Location	Company	Description	Sample code	Sample date	BBP	DCHP	DEHP	DOP
Surface water					Concentrations in µg/l			
Karlsruhe	TZW	Rhine (D)	W.OW.1.KRL.HOR.F	8-3-99	0,067	0,020	0,342	0,015
Köln	GEW	Site 1	W.OW.1.KO1.HOR.F	8-3-99	0,072	< 0,007	0,521	< 0,005
Köln	GEW	Site 2	W.OW.1.KO2.HOR.F	8-3-99	0,025	0,009	0,200	< 0,002
Köln	GEW	Site 3	W.OW.1.KO3.HOR.F	8-3-99	0,082	< 0,003	0,311	< 0,003
Köln	GEW	Site 4	W.OW.1.KO4.HOR.F	8-3-99	0,068	0,015	0,147	0,016
Köln	GEW	Site 5	W.OW.1.KO5.HOR.F	8-3-99	0,099	< 0,004	0,427	< 0,005
Köln	GEW	Site 6	W.OW.1.KO6.HOR.F	8-3-99	0,042	< 0,003	0,326	< 0,002
Lobith	RIZA	German-Dutch border	A.OW.1.LOB.HOR.F	15-3-99	0,020	< -0,001	0,580	0,016
Lekkanaal	WRK	Lekkanaal: inlet	W.OW.1.LKN.HOR.F	8-3-99	0,033	0,008	0,242	< 0,004
Andijk	PWN	Inlet at IJsselmeer	W.OW.1.AND.HOR.F	8-3-99	0,486	< 0,006	0,431	< 0,005
Twentekanaal	WMO	Inlet WMO	W.OW.1.WMO.HOR.F	8-3-99	0,102	0,009	? 0,406	? 0,014
				minimum	0,020	-0,001	0,147	0,002
				maximum	0,486	0,020	0,580	0,016
				mediaan	0,068	0,007	0,334	0,005
				90 percentiel	0,102	0,015	0,527	0,016
Proceswater								
Lekkanaal	WRK	Finished product	W.LW.1.LKN.HOR.O	8-3-99	0,032	< 0,004	0,257	< 0,002
Lekkerkerk	WZHO	GLS PF 99A	W.OW.1.WZ1.HOR.F	8-3-99	0,042	< 0,001	< 0,147	< 0,003
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.1.WZ2.HOR.F	8-3-99	0,037	0,000	< 0,072	< 0,005
Leiduin	GWA	After infitration (a.i.)	W.LW.1.GW2.HOR.O	8-3-99	< 0,011	< 0,002	< 0,080	< 0,008
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.1.GW3.HOR.O	8-3-99	< 0,008	< 0,001	< -0,017	< 0,004
Leiduin	GWA	After active carbon filtration (a.c.)	W.LW.1.GW4.HOR.O	8-3-99	< 0,019	< -0,001	< 0,085	< 0,008
Drinkwater								
Leiduin	GWA	Drinking water	W.LW.1.GW1.HOR.O	8-3-99	0,058	0,000	0,243	0,010
Andijk	PWN	Drinking water	W.LW.1.AND.HOR.O	8-3-99	0,486	< 0,006	0,431	< 0,005
Twentekanaal	WMO	Drinking water	W.LW.1.WMO.HOR.O	8-3-99	< 0,012	< -0,001	< 0,142	< 0,003
				minimum	0,008	-0,001	-0,017	0,002
				maximum	0,486	0,006	0,431	0,010

Phase 2 **June 1999** **Phtalates analysis Meuse**

Location	Company	Description	Sample code	Sample date	DMP	DEP	DPP	DMPP	DBP
					Concentration in µg/l				
Surface water									
Remilly	BIWM	French-Belgian border	W.OW.2.REM.FTA.F	28-6-99	< 0,011	< 0,209	< 0,000	0,375	0,159
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.2.TAI.FTA.F	28-6-99	< 0,006	< -0,040	< 0,000	0,354	< 0,094
Namêche	AWW	Meuse	W.OW.2.NAM.FTA.F	28-6-99	< 0,003	< 0,043	< 0,000	0,451	0,204
Liège	AWW	Albert kanaal	W.OW.2.LUI.FTA.F	28-6-99	< 0,000	< 0,140	< 0,000	0,428	0,373
Eijsden	RIZA	Belgian-Dutch border	A.OW.2.EYS.FTA.F	7/13/99	0,182	0,624	0,007	0,883	1,335
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.2.WPH.FTA.F	30-6-99	< -0,005	< -0,076	< 0,000	0,199	< 0,118
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.2.WPR.FTA.F	30-6-99	< 0,035	< 0,122	< 0,000	0,637	0,361
Belfeld	RW-DL	Meuse (NL)	W.OW.2.BEL.FTA.F	29-6-99	0,069	< 0,142	< 0,000	0,708	0,298
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.2.KEI.FTA.F	29-6-99	< 0,007	< 0,045	< 0,000	2,418	0,158
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.2.WBB.FTA.F	28-6-99	< 0,021	< 0,207	< 0,000	0,647	0,273
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.2.BRA.FTA.F	28-6-99	0,187	2,631	0,022	2,779	1,884
				minimum	-0,005	-0,076	0,000	0,199	0,094
				maximum	0,187	2,631	0,022	2,779	1,884
				mediaan	0,011	0,140	0,000	0,637	0,273
				90 percentiel	0,182	0,624	0,007	2,418	1,335
Process water									
Petrusplaat	WBB	Finished product	W.LW.2.WBB.FTA.O	28-6-99	< 0,005	< 0,294	< 0,000	0,996	0,475
Scheveningen	DZH	Collected after extraction	W.LW.2.DZ2.FTA.O	29-6-99	< 0,004	< 0,216	< 0,000	0,282	< 0,134
Ouddorp	Delta	Collected process water	W.OW.2.OUD.FTA.F	29-6-99	< 0,008	< 0,153	< 0,000	0,177	0,178
Drinking water									
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.2.DZ1.FTA.O	29-6-99	< -0,007	< -0,023	< 0,000	0,454	< 0,092
Ouddorp	Delta	Drinking water	W.LW.2.OUD.FTA.O	29-6-99	< 0,015	< -0,061	< 0,000	< 0,069	< 0,058
Braakman	Delta	Drinking water	W.LW.2.BRA.FTA.O	29-6-99	< 0,015	< -0,061	< 0,000	< 0,069	< 0,058
Kralingen	WBE	Drinking water	W.LW.2.WB1.FTA.O	30-6-99	< -0,003	< -0,079	< 0,000	< 0,085	< 0,101
Berenplaat	WBE	Drinking water	W.LW.2.WB2.FTA.O	30-6-99	< -0,001	< -0,032	< 0,000	0,384	< 0,093
				minimum	-0,007	-0,079	0,000	0,069	0,058
				maximum	0,015	0,294	0,000	0,996	0,475

Legend: ? = recovery lower than 40%

Phase 2 **June 1999** **Phtalates analysis Meuse**

continued

Location	Company	Description	Sample code	Sample date	BBP	DCHP	DEHP	DOP
					Concentration in µg/l			
Surface water								
Remilly	BIWM	French-Belgian border	W.OW.2.REM.FTA.F	28-6-99	0,029	< -0,003	0,303	< 0,000
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.2.TAI.FTA.F	28-6-99	< 0,018	< -0,003	1,556	< 0,000
Namêche	AWW	Meuse	W.OW.2.NAM.FTA.F	28-6-99	0,046	< -0,003	0,329	< 0,000
Liège	AWW	Albert kanaal	W.OW.2.LUI.FTA.F	28-6-99	0,090	< -0,003	0,456	< 0,000
Eijsden	RIZA	Belgian-Dutch border	A.OW.2.EYS.FTA.F	13-7-99	0,572	0,008	? 0,620	? 0,035
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.2.WPH.FTA.F	30-6-99	0,077	< -0,003	0,343	< 0,000
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.2.WPR.FTA.F	30-6-99	0,158	0,061	? 0,317	< 0,000
Belfeld	RW-DL	Meuse (NL)	W.OW.2.BEL.FTA.F	29-6-99	0,068	0,053	0,370	< 0,000
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.2.KEI.FTA.F	29-6-99	0,075	< 0,001	0,305	< 0,007
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.2.WBB.FTA.F	28-6-99	0,084	< -0,003	0,219	< 0,000
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.2.BRA.FTA.F	28-6-99	1,995	< -0,003	200,348	4,644
				minimum	0,018	-0,003	0,219	0,000
				maximum	1,995	0,061	200,348	4,644
				mediaan	0,077	-0,003	0,343	0,000
				90 percentiel	0,572	0,053	1,556	0,035
Process water								
Petrusplaat	WBB	Finished product	W.LW.2.WBB.FTA.O	28-6-99	0,136	< 0,001	0,432	< 0,000
Scheveningen	DZH	Collected after extraction	W.LW.2.DZ2.FTA.O	29-6-99	0,036	< -0,003	0,361	< 0,000
Ouddorp	Delta	Collected process water	W.OW.2.OUD.FTA.F	29-6-99	0,049	< -0,003	0,318	< 0,000
Drinking water								
Scheveningen	DZH	Collected after filtration, drinking water	W.LW.2.DZ1.FTA.O	29-6-99	< 0,017	< -0,003	< 0,088	< 0,000
Ouddorp	Delta	Outgoing water	W.LW.2.OUD.FTA.O	29-6-99	0,031	0,019	0,118	< 0,000
Braakman	Delta	Outgoing water	W.LW.2.BRA.FTA.O	29-6-99	0,031	0,019	0,118	< 0,000
Kralingen	WBE	Drinking water	W.LW.2.WB1.FTA.O	30-6-99	< 0,014	< -0,003	0,196	< 0,000
Berenplaat	WBE	Drinking water	W.LW.2.WB2.FTA.O	30-6-99	< 0,009	< -0,003	< 0,060	< 0,000
				minimum	0,009	-0,003	0,060	0,000
Legend: ? = recovery lower than 40%				maximum	0,136	0,019	0,432	0,000

Phase 2 **June 1999** **Phtalates analysis Rhine**

Location	Company	Description	Sample code	Sample date	DMP	DEP	DPP	DMPP	DBP
					Concentration in µg/l				
Surface water									
Lobith	RIZA	German-Dutch border	A.OW.2.LOB.FTA.F	5-7-99	0,102	0,100	< 0,000	0,300	0,290
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.2.LKN.FTA.F	30-6-99	< 0,021	< 0,175	< 0,000	0,193	0,211
Andijk	PWN	Inlet at IJsselmeer	W.OW.2.AND.FTA.F	28-6-99	< 0,005	< 0,075	< 0,000	0,355	0,471
Twentekanaal	WMO	Inlet WMO	W.OW.2.WMO.FTA.F	28-6-99	< 0,006	< 0,093	< 0,000	0,594	0,227
				minimum	0,005	0,075	0,000	0,193	0,211
				maximum	0,102	0,175	0,000	0,594	0,471
				mediaan	0,013	0,097	0,000	0,328	0,258
				90 percentiel	0,077	0,152	0,000	0,523	0,417
Process water									
Nieuwegein	WRK	Finished product	W.LW.2.LKN.FTA.O	30-6-99	< 0,003	< -0,042	< 0,000	0,219	< 0,091
Lekkerkerk	WZHO	GLS PF 99	W.OW.2.WZ1.FTA.F	28-6-99	< 0,026	< 0,175	0,026	0,402	0,403
Nieuw Lekkerlan	WZHO	GPU PE 99B (De Put)	W.OW.2.WZ2.FTA.F	28-6-99	< -0,004	< -0,021	< 0,000	< 0,096	0,143
Leiduin	GWA	After infitration (a.i.)	W.LW.2.GW2.FTA.O	29-6-99	< -0,005	< -0,008	< 0,000	0,140	< 0,105
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.2.GW3.FTA.O	29-6-99	< -0,003	< 0,010	< 0,000	0,422	< 0,068
Drinking water									
Leiduin	GWA	Drinking water	W.LW.2.GW1.FTA.O	29-6-99	< -0,004	< 0,050	< 0,000	0,418	0,143
Weesperkarspel	GWA	Drinking water	W.LW.2.GW4.FTA.O	29-6-99	< -0,005	< 0,005	< 0,000	0,411	< 0,044
Andijk	PWN	Drinking water	W.LW.2.AND.FTA.O	28-6-99	< -0,004	< 0,057	< 0,000	0,488	0,189
Twentekanaal	WMO	Drinking water	W.LW.2.WMO.FTA.O	28-6-99	< 0,018	< 0,233	< 0,000	0,396	0,235
				minimum	-0,005	-0,042	0,000	0,096	0,044
				maximum	0,026	0,233	0,026	0,488	0,403

Legend: ? = recovery lower than 40%

Phase 2 **June 1999** **Phtalates analysis Rhine**

continued

Location	Company	Description	Sample code	Sample date	BBP	DCHP	DEHP	DOP
					Concentration in µg/l			
Surface water								
Lobith	RIZA	German-Dutch border	A.OW.2.LOB.FTA.F	5-7-99	0,093	0,009	0,919	< 0,000
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.2.LKN.FTA.F	30-6-99	0,061	< -0,003	0,275	< 0,000
Andijk	PWN	Inlet at IJsselmeer	W.OW.2.AND.FTA.F	28-6-99	0,314	< -0,003	0,438	< 0,000
Twentekanaal	WMO	Inlet WMO	W.OW.2.WMO.FTA.F	28-6-99	0,125	< -0,003	0,360	< 0,000
				minimum	0,061	-0,003	0,275	0,000
				maximum	0,314	0,009	0,919	0,000
				mediaan	0,109	-0,003	0,399	0,000
				90 percentiel	0,257	0,005	0,774	0,000
Process water								
Nieuwegein	WRK	Finished product	W.LW.2.LKN.FTA.O	30-6-99	< 0,020	< -0,003	< 0,060	< 0,000
Lekkerkerk	WZHO	GLS PF 99	W.OW.2.WZ1.FTA.F	28-6-99	0,259	0,023	0,418	< 0,000
Nieuw Lekkerlan	WZHO	GPU PE 99B (De Put)	W.OW.2.WZ2.FTA.F	28-6-99	0,051	< -0,003	< 0,089	< 0,000
Leiduin	GWA	After infitration (a.i.)	W.LW.2.GW2.FTA.O	29-6-99	0,039	< -0,003	< 0,100	< 0,000
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.2.GW3.FTA.O	29-6-99	< 0,017	< -0,003	< 0,094	< 0,000
Drinking water								
Leiduin	GWA	Drinking water	W.LW.2.GW1.FTA.O	29-6-99	0,036	< 0,000	0,142	< 0,000
Weesperkarspel	GWA	Drinking water	W.LW.2.GW4.FTA.O	29-6-99	< 0,010	< -0,003	< 0,085	< 0,000
Andijk	PWN	Drinking water	W.LW.2.AND.FTA.O	28-6-99	0,106	< -0,003	0,646	< 0,000
Twentekanaal	WMO	Drinking water	W.LW.2.WMO.FTA.O	28-6-99	0,096	< 0,001	0,290	< 0,000
				minimum	0,010	-0,003	0,060	0,000
				maximum	0,259	0,023	0,646	0,000

Legend: ? = recovery lower than 40%

Phase 3 **September 1999** **Phthalates analysis Meuse**

Location	Company	Description	Sample code	Sample date	DMP	DEP	DPP	DMPP	DBP
Surface water					Concentrations in µg/l				
Remilly	BIWM	French-Belgian border	W.OW.3.REM.FTA.F	20-9-99	0,010	1,559	< 0,000	0,478	0,255
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.3.TAI.FTA.F	20-9-99	0,014	2,322	< 0,000	0,122	0,232
Namêche	AWW	Meuse	W.OW.3.NAM.FTA.F	20-9-99	0,009	1,413	< 0,000	0,544	0,215
Liège	AWW	Albert kanaal	W.OW.3.LUI.FTA.F	20-9-99	0,014	1,160	< 0,000	0,440	0,356
Eijsden	RIZA	Belgian-Dutch border	A.OW.3.EYS.FTA.F	28-9-99	0,022	< 0,297	< 0,000	0,002	0,098
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.3.WPH.FTA.F	21-9-99	0,004	< -0,094	< 0,000	0,651	0,111
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.3.WPR.FTA.F	21-9-99	0,008	2,278	< 0,000	1,105	0,322
Belfeld	RW-DL	Meuse (NL)	W.OW.3.BEL.FTA.F	21-9-99	0,105	< -0,121	< 0,000	0,782	0,160
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.3.KEI.FTA.F	21-9-99	0,007	1,294	< 0,000	0,551	0,067
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.3.WBB.FTA.F	20-9-99	0,010	2,168	< 0,000	0,435	0,192
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.3.BRA.FTA.F	20-9-99	0,017	2,296	< 0,000	0,844	0,579
				minimum	0,004	-0,121	0,000	0,002	0,067
				maximum	0,105	2,322	0,000	1,105	0,579
				mediaan	0,010	1,413	0,000	0,544	0,215
				90 percentiel	0,022	2,296	0,000	0,844	0,356
Process water									
Petrusplaat	WBB	Finished product	W.LW.3.WBB.FTA.O	20-9-99	< 0,001	< -0,018	< 0,000	< 0,000	0,276
Scheveningen	DZH	Collected after extraction	W.LW.3.DZ2.FTA.O	21-9-99	0,008	< -0,154	< 0,000	0,766	0,942
Ouddorp	Delta	Collected process water	W.OW.3.OUD.FTA.F	21-9-99	0,013	0,788	< 0,000	0,102	0,042
Drinking water									
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.3.DZ1.FTA.O	21-9-99	< 0,001	< -0,149	< 0,000	0,560	0,971
Ouddorp	Delta	Outgoing water	W.LW.3.OUD.FTA.O	21-9-99	< 0,001	< -0,262	< 0,000	0,137	0,052
Braakman	Delta	Outgoing water	W.LW.3.BRA.FTA.O	20-9-99	0,096	1,570	< 0,000	0,099	0,252
Kralingen	WBE	Drinking water	W.LW.3.WB1.FTA.O	22-9-99	< -0,001	< -0,251	< 0,000	0,110	< -0,014
Berenplaat	WBE	Drinking water	W.LW.3.WB2.FTA.O	22-9-99	0,009	< -0,144	< 0,000	1,322	1,104
				minimum	-0,001	-0,262	0,000	0,000	-0,014
				maximum	0,096	1,570	0,000	1,322	1,104

Legend: ? = recovery lower than 40%

Phase 3 **September 1999** **Phthalates analysis Meuse**

continued

Location	Company	Description	Sample code	Sample date	BBP	DCHP	DEHP	DOP
Surface water					Concentrations in µg/l			
Remilly	BIWM	French-Belgian border	W.OW.3.REM.FTA.F	20-9-99	0,019	0,002	0,184	0,003
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.3.TAI.FTA.F	20-9-99	0,025	0,003	0,171	0,003
Namêche	AWW	Meuse	W.OW.3.NAM.FTA.F	20-9-99	0,046	< 0,000	0,650	0,005
Liège	AWW	Albert kanaal	W.OW.3.LUI.FTA.F	20-9-99	0,034	0,006	0,249	? 0,003
Eijsden	RIZA	Belgian-Dutch border	A.OW.3.EYS.FTA.F	28-9-99	0,025	0,007	0,357	< 0,010
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.3.WPH.FTA.F	21-9-99	0,029	< 0,000	0,201	0,001
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.3.WPR.FTA.F	21-9-99	0,018	0,002	0,156	0,005
Belfeld	RW-DL	Meuse (NL)	W.OW.3.BEL.FTA.F	21-9-99	0,053	< 0,000	0,647	? 0,028
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.3.KEI.FTA.F	21-9-99	0,012	0,003	0,097	0,003
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.3.WBB.FTA.F	20-9-99	0,053	0,008	0,280	0,002
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.3.BRA.FTA.F	20-9-99	0,029	0,005	0,152	0,005
				minimum	0,012	0,000	0,097	0,001
				maximum	0,053	0,008	0,650	0,028
				mediaan	0,029	0,003	0,201	0,003
				90 percentiel	0,053	0,007	0,647	0,010
Process water								
Petrusplaat	WBB	Finished product	W.LW.3.WBB.FTA.O	20-9-99	0,049	0,005	< 0,175	< 0,008
Scheveningen	DZH	Collected after extraction	W.LW.3.DZ2.FTA.O	21-9-99	0,255	0,002	0,219	< -0,003
Ouddorp	Delta	Collected process water	W.OW.3.OUD.FTA.F	21-9-99	0,018	0,004	0,130	< -0,002
Drinking water								
Scheveningen	DZH	Collected after filtration, drinking water	W.LW.3.DZ1.FTA.O	21-9-99	0,248	0,003	0,222	< -0,002
Ouddorp	Delta	Outgoing water	W.LW.3.OUD.FTA.O	21-9-99	0,029	0,001	0,271	0,002
Braakman	Delta	Outgoing water	W.LW.3.BRA.FTA.O	20-9-99	0,029	0,005	0,152	0,005
Kralingen	WBE	Drinking water	W.LW.3.WB1.FTA.O	22-9-99	0,010	< 0,000	0,152	< -0,002
Berenplaat	WBE	Drinking water	W.LW.3.WB2.FTA.O	22-9-99	0,273	0,004	? 0,404	? 0,008
				minimum	0,010	0,000	0,130	-0,003
				maximum	0,273	0,005	0,404	0,008

Legend: ? = recovery lower than 40%

Phase 3 **September 1999** **Phthalates analysis Rhine**

Location	Company	Description	Sample code	Sample date	DMP	DEP	DPP	DMPP	DBP
					Concentrations in µg/l				
Surface water									
Lobith	RIZA	German-Dutch border	A.OW.3.LOB.FTA.F	10-4-99	0,028	< 0,011	< 0,000	< 0,000	0,204
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.3.LKN.FTA.F	22-9-99	0,037	< -0,134	< 0,000	0,462	0,152
A'dam-Rijnkanaal	GWA	Inlet Weesperkarspel	W.OW.3.GW1.FTA.O	22-9-99	0,011	< -0,149	< 0,000	0,482	0,044
Andijk	PWN	Inlet at IJsselmeer	W.OW.3.AND.FTA.F	20-9-99	0,011	0,694	< 0,000	0,055	0,099
Twentekanaal	WMO	Inlet WMO	W.OW.3.WMO.FTA.F	20-9-99	0,017	0,193	< 0,000	0,147	0,410
				minimum	0,011	-0,149	0,000	0,000	0,044
				maximum	0,037	0,694	0,000	0,482	0,410
				mediaan	0,017	0,011	0,000	0,147	0,152
				90 percentiel	0,033	0,494	0,000	0,474	0,327
Process water									
Nieuwegein	WRK	Finished product	W.LW.3.LKN.FTA.O	22-9-99	0,008	< -0,186	< 0,000	0,198	0,023
Lekkerkerk	WZHO	GLS PF 99	W.OW.3.WZ1.FTA.F	20-9-99	0,018	1,194	< 0,000	1,026	0,135
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.3.WZ2.FTA.F	20-9-99	0,018	0,976	< 0,000	0,126	0,032
Leiduin	GWA	After infiltration (a.i.)	W.LW.3.GW2.FTA.O	22-9-99	< 0,000	< -0,221	< 0,000	0,306	0,055
Drinking water									
Leiduin	GWA	Drinking water	W.LW.3.GW1.FTA.O	22-9-99	< 0,001	< -0,150	< 0,000	0,034	< -0,017
Weesperkarspel	GWA	Drinking water	W.LW.3.GW2.FTA.O	22-9-99	< 0,002	< -0,252	< 0,000	0,213	0,037
Andijk	PWN	Drinking water	W.LW.3.AND.FTA.O	20-9-99	< 0,002	2,103	< 0,000	0,559	0,586
Twentekanaal	WMO	Drinking water	W.LW.3.WMO.FTA.O	20-9-99	0,004	1,425	< 0,000	0,233	0,424
				minimum	0,000	-0,252	0,000	0,034	-0,017
				maximum	0,018	2,103	0,000	1,026	0,586

Legend: ? = recovery lower than 40%

Phase 3 **September 1999** **Phthalates analysis Rhine**

continued

Location	Company	Description	Sample code	Sample date	BBP	DCHP	DEHP	DOP
Surface water					Concentrations in µg/l			
Lobith	RIZA	German-Dutch border	A.OW.3.LOB.FTA.F	4-10-99	0,048	< 0,000	0,541	< 0,007
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.3.LKN.FTA.F	22-9-99	0,087	0,003	0,249	0,003
A'dam-Rijnkanaal	GWA	Inlet Weesperkarspel	W.OW.3.GW1.FTA.O	22-9-99	0,026	0,001	0,242	0,008
Andijk	PWN	Inlet at IJsselmeer	W.OW.3.AND.FTA.F	20-9-99	0,058	0,007	? 0,487	? 0,007
Twentekanaal	WMO	Inlet WMO	W.OW.3.WMO.FTA.F	20-9-99	0,359	0,003	0,200	0,003
				minimum	0,026	0,000	0,200	0,003
				maximum	0,359	0,007	0,541	0,008
				mediaan	0,058	0,003	0,249	0,007
				90 percentiel	0,250	0,006	0,519	0,008
Process water								
Nieuwegein	WRK	Finished product	W.LW.3.LKN.FTA.O	22-9-99	0,012	< 0,000	0,095	< -0,001
Lekkerkerk	WZHO	GLS PF 99	W.OW.3.WZ1.FTA.F	20-9-99	0,018	0,001	0,102	< -0,001
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.3.WZ2.FTA.F	20-9-99	0,028	< 0,000	0,406	0,002
Leiduin	GWA	After infitration (a.i.)	W.LW.3.GW2.FTA.O	22-9-99	0,007	< 0,000	0,116	0,009
Drinking water								
Leiduin	GWA	Drinking water	W.LW.3.GW1.FTA.O	22-9-99	0,005	< 0,000	0,056	< -0,001
Weesperkarspel	GWA	Drinking water	W.LW.3.GW3.FTA.O	22-9-99	0,009	< 0,000	? 0,178	? 0,001
Andijk	PWN	Drinking water	W.LW.3.AND.FTA.O	20-9-99	0,359	0,003	0,200	0,003
Twentekanaal	WMO	Drinking water	W.LW.3.WMO.FTA.O	20-9-99	0,044	0,003	0,158	0,010
				minimum	0,005	0,000	0,056	-0,001
				maximum	0,359	0,003	0,406	0,010

Legend: ? = recovery lower than 40%

Phase 1 **March 1999** **Alkylphenol Polyethoxylates analysis Meuse**

Location	Company	Description	Sample code	Sample date	nonylphenol-ethoxylaten	octylphenol-ethoxylaten	nonylphenol	octylphenol
Surface water					Concentration in µg/l			
Remilly	BIWM	French-Belgian border	W.OW.1.REM.APE.F	8-3-99	< 0,860	< 0,920	< 0,710	< 0,320
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.1.TAI.APE.F	8-3-99	< 0,110	< 0,340	< 0,330	< 0,110
Namêche	AWW	Meuse	W.OW.1.NAM.APE.F	8-3-99	< 0,460	< 0,510	< 0,490	< 0,200
Liège	AWW	Albert kanaal	W.OW.1.LUI.APE.F	8-3-99	< 0,200	< 0,250	< 0,230	< 0,090
Eijsden	RIZA	Belgian-Dutch border	A.OW.1.EYS.APE.F	23-3-99	< 0,870	< 0,410	< 0,440	< 0,160
Belfeld	RW-DL	Meuse (NL)	W.OW.1.BEL.APE.F	9-3-99	< 0,320	< 0,320	< 0,310	< 0,100
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.1.KEI.APE.F	9-3-99	< 0,460	< 0,460	< 0,400	< 0,130
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.1.WBB.APE.F	9-3-99	< 1,130	< 0,560	< 0,530	< 0,660
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.1.BRA.APE.F	8-3-99	< 0,130	< 0,360	< 0,340	< 0,130
				minimum	0,110	0,250	0,230	0,090
				maximum	1,130	0,920	0,710	0,660
				mediaan	0,460	0,410	0,400	0,130
				90 percentiel	0,922	0,632	0,566	0,388
Proceswater								
Petrusplaat	WBB	Finished product	W.LW.1.WBB.APE.O	9-3-99	< 0,190	< 0,190	< 0,180	< 0,090
Scheveningen	DZH	Collected after extraction	W.LW.1.DZ2.APE.O	8-3-99	1,500	< 0,240	< 0,220	< 0,080
Drinking water								
Scheveningen	DZH	Collected after filtration	W.LW.1.DZ1.APE.O	8-3-99	< 0,100	< 0,300	< 0,290	< 0,100
				minimum	0,100	0,190	0,180	0,080
				maximum	1,500	0,300	0,290	0,100

Legend: <* = results are an indication for they are beyond the levels of certain quantification

Phase 1 March 1999 Alkylphenol Polyethoxylates analysis Rhine

Location	Company	Description	Sample code	Sample date	nonylphenol-ethoxylaten	octylphenol-ethoxylaten	nonylphenol	octylphenol
Surface water					Concentration in µg/l			
Karlsruhe	TZW	Rhine (D)	W.OW.1.KRL.APE.F	8-3-99	< 0,170	< 0,360	< 0,340	< 0,130
Köln	GEW	Site 1	W.OW.1.KO1.APE.F	8-3-99	< 0,160	< 0,360	< 0,340	< 0,130
Köln	GEW	Site 2	W.OW.1.KO2.APE.F	8-3-99	< 0,280	< 0,600	< 0,580	< 0,240
Köln	GEW	Site 3	W.OW.1.KO3.APE.F	8-3-99	< 0,240	< 0,510	< 0,500	< 0,220
Köln	GEW	Site 4	W.OW.1.KO4.APE.F	8-3-99	< 0,150	< 0,350	< 0,340	< 0,130
Köln	GEW	Site 5	W.OW.1.KO5.APE.F	8-3-99	< 0,200	< 0,250	< 0,230	< 0,090
Köln	GEW	Site 6	W.OW.1.KO6.APE.F	8-3-99	< 0,210	< 0,270	< 0,230	< 0,110
Lobith	RIZA	German-Dutch border	W.OW.1.LOB.APE.F	15-3-99	< 1,260	< 0,680	< 0,440	< 0,270
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.1.LKN.APE.F	8-3-99	< 0,280	< 0,410	< 0,400	< 0,160
Andijk	PWN	Inlet at IJsselmeer	W.OW.1.AND.APE.F	8-3-99	< 0,670	< 0,500	< 0,470	< 0,180
Twentekanaal	WMO	Inlet WMO	W.OW.1.WMO.APE.F	8-3-99	< 0,710	< 0,420	< 0,390	< 0,150
				minimum	0,150	0,250	0,230	0,090
				maximum	1,260	0,680	0,580	0,270
				mediaan	0,240	0,410	0,390	0,150
				90 percentiel	0,710	0,600	0,500	0,240
Proceswater								
Nieuwegein	WRK	Finished product	W.LW.1.LKN.APE.O	8-3-99	4,500	< 0,700	< 0,660	< 0,250
Lekkerkerk	WZHO	GLS PF 99	W.OW.1.WZ1.APE.F	8-3-99	< 0,570	< 0,570	< 0,530	< 0,200
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.1.WZ2.APE.F	8-3-99	< 0,430	< 0,430	< 0,410	< 0,150
Leiduin	GWA	After infitration (a.i.)	W.LW.1.GW2.APE.O	8-3-99	< 1,000	< 0,570	< 0,540	< 0,180
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.1.GW3.APE.O	8-3-99	< 0,800	< 0,540	< 0,510	< 0,190
Leiduin	GWA	After active carbon filtration (a.c.)	W.LW.1.GW4.APE.O	8-3-99	< 0,860	< 0,670	< 0,630	< 0,230
Drinkwater								
Leiduin	GWA	Drinking water	W.LW.1.GW1.APE.O	8-3-99	< 1,400	< 0,500	< 0,470	< 0,180
Andijk	PWN	Drinking water	W.LW.1.AND.APE.O	8-3-99	2,020	< 0,470	< 0,440	< 0,170
WMO	WMO	Drinking water	W.LW.1.WMO.APE.O	8-3-99	< 0,600	< 0,600	< 0,560	< 0,210
				minimum	0,430	0,430	0,410	0,150
				maximum	4,500	0,700	0,660	0,250

Legend: <* = results are an indication for they are beyond the levels of certain quantification

Phase 2 **June 1999** **Alkylphenol ethoxylates analysis Meuse**

Location	Company	Description	Sample code	Sample date	nonylphenol-ethoxylaten	octylphenol-ethoxylaten	nonylphenol	octylphenol
Surface water						Concentration in µg/l		
Remilly	BIWM	French-Belgian border	W.OW.2.REM.APE.F	28-6-99	< 0,385	< 0,233	< 0,222	< 0,094
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.2.TAI.APE.F	28-6-99	< 0,512	< 0,309	< 0,295	< 0,125
Namêche	AWW	Meuse	W.OW.2.NAM.APE.F	28-6-99	< 0,467	< 0,282	< 0,269	< 0,114
Liège	AWW	Albert kanaal	W.OW.2.LUI.APE.F	28-6-99	< 0,191	< 0,170	< 0,163	< 0,069
Eijsden	RIZA	Belgian-Dutch border	A.OW.2.EYS.APE.F	13-7-99	< 0,797	< 0,224	< 0,217	< 0,087
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.2.WPH.APE.F	30-6-99	< 1,745	< 1,557	< 1,486	< 0,628
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.2.WPR.APE.F	30-6-99	2,450	< 0,371	< 0,354	< 0,150
Belfeld	RW-DL	Meuse (NL)	W.OW.2.BEL.APE.F	29-6-99	2,000	< 0,300	< 0,287	< 0,121
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.2.KEI.APE.F	29-6-99	< 0,365	< 0,221	< 0,211	< 0,089
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.2.WBB.APE.F	28-6-99	< 0,544	< 0,329	< 0,314	< 0,133
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.2.BRA.APE.F	28-6-99	< 0,609	< 0,368	< 0,351	< 0,148
				minimum	0,191	0,170	0,163	0,069
				maximum	2,450	1,557	1,486	0,628
				mediaan	0,544	0,300	0,287	0,121
				90 percentiel	2,000	0,371	0,354	0,150
Process water								
Petrusplaat	WBB	Finished product	W.LW.2.WBB.APE.O	28-6-99	< -	< 0,247	< 0,236	< 0,100
Scheveningen	DZH	Collected after extraction	W.LW.2.DZ2.APE.O	29-6-99	< 0,324	< 0,196	< 0,187	< 0,079
Ouddorp	Delta	Collected process water after infiltration	W.OW.2.OUD.APE.F	29-6-99	< 0,500	< 0,342	< 0,327	< 0,138
Drinking water								
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.2.DZ1.APE.O	29-6-99	< 0,572	< 0,345	< 0,330	< 0,139
Ouddorp	Delta	Drinking water	W.LW.2.OUD.APE.O	29-6-99	< 0,484	< 0,292	< 0,279	< 0,118
Braakman	Delta	Drinking water	W.LW.2.BRA.APE.O	29-6-99	< 0,371	< 0,224	< 0,214	< 0,091
Kralingen	WBE	Drinking water	W.LW.2.WB1.APE.O	30-6-99	2,103	< 0,239	< 0,247	< 0,097
Berenplaat	WBE	Drinking water	W.LW.2.WB2.APE.O	30-6-99	2,079	< 0,266	< 0,254	< 0,107
				minimum	0,324	0,196	0,187	0,079
Legend: <* = results are an indication for they are beyond the levels of certain quantification				maximum	2,103	0,345	0,330	0,139

Phase 2 June 1999 Alkylphenol ethoxylates analysis Rhine

Location	Company	Description	Sample code	Sample date	nonylphenol-ethoxylaten	octylphenol-ethoxylaten	nonylphenol	octylphenol
Surface water					Concentration in µg/l			
Lobith	RIZA	German-Dutch border	A.OW.2.LOB.APE.F	5-7-99	2,596	< 0,373	< 0,362	< 0,145
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.2.LKN.APE.F	30-6-99	1,867	< 0,360	< 0,343	< 0,145
Andijk	PWN	Inlet at IJsselmeer	W.OW.2.AND.APE.F	28-6-99	< 0,303	< 0,183	< 0,175	< 0,074
Twentekanaal	WMO	Inlet WMO	W.OW.2.WMO.APE.F	28-6-99	< 0,498	< 0,301	< 0,287	< 0,121
				minimum	0,303	0,183	0,175	0,074
				maximum	2,596	0,373	0,362	0,145
				mediaan	1,182	0,330	0,315	0,133
				90 percentiel	2,377	0,369	0,356	0,145
Process water								
Nieuwegein	WRK	Finished product	W.LW.2.LKN.APE.O	30-6-99	< 0,370	< 0,224	< 0,214	< 0,090
Lekkerkerk	WZHO	GLS PF 99	W.OW.2.WZ1.APE.F	28-6-99	< 2,514	< 0,453	< 0,432	< 0,183
Nieuw Lekkerlan	WZHO	GPU PE 99B (De Put)	W.OW.2.WZ2.APE.F	28-6-99	1,592	< 0,288	< 0,275	< 0,116
Leiduin	GWA	After infitration (a.i.)	W.LW.2.GW2.APE.O	29-6-99	< 0,292	< 0,177	< 0,169	< 0,071
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.2.GW3.APE.O	29-6-99	< 0,488	< 0,295	< 0,282	< 0,119
Drinking water								
Leiduin	GWA	Drinking water	W.LW.2.GW1.APE.O	29-6-99	< 0,319	< 0,193	< 0,184	< 0,078
Weesperkarspel	GWA	Drinking water	W.LW.2.GW4.APE.O	29-6-99	< 0,382	< 0,231	< 0,220	< 0,093
Andijk	PWN	Drinking water	W.LW.2.AND.APE.O	28-6-99	< 0,596	< 0,360	< 0,344	< 0,145
Twentekanaal	WMO	Drinking water	W.LW.2.WMO.APE.O	28-6-99	< 0,499	< 0,302	< 0,288	< 0,122
				minimum	0,292	0,177	0,169	0,071
				maximum	2,514	0,453	0,432	0,183

Legend: <* = results are an indication for they are beyond the levels of certain quantification

Phase 3 September 1999 Alkylphenol ethoxylates analysis Meuse

Location	Company		Sample code	Sample date	Nonylphenol-ethoxylates	Octylphenol-ethoxylates	Nonylphenol	Octylphenol
Surface water							Concentration in µg/l	
Remilly	BIWM	French-Belgian border	W.OW.3.REM.APE.F	20-9-99	< 0,328	< 0,172	< 0,181	< 0,075
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.3.TAI.APE.F	20-9-99	< 0,352	< 0,185	< 0,194	< 0,080
Namêche	AWW	Meuse	W.OW.3.NAM.APE.F	20-9-99	< 0,401	< 0,211	< 0,221	< 0,091
Liège	AWW	Albert kanaal	W.OW.3.LUI.APE.F	20-9-99	< 0,309	< 0,162	< 0,170	< 0,070
Eijsden	RIZA	Belgian-Dutch border	A.OW.3.EYS.APE.F	28-9-99	< 0,395	< 0,208	< 0,220	< 0,090
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.3.WPH.APE.F	21-9-99	< 0,350	< 0,184	< 0,193	< 0,080
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.3.WPR.APE.F	21-9-99	< 0,450	< 0,237	< 0,248	< 0,103
Belfeld	RW-DL	Meuse (NL)	W.OW.3.BEL.APE.F	21-9-99	< 0,471	< 0,248	< 0,260	< 0,107
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.3.KEI.APE.F	21-9-99	< 0,495	< 0,260	< 0,273	< 0,113
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.3.WBB.APE.F	20-9-99	< 0,327	< 0,172	< 0,180	< 0,075
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.3.BRA.APE.F	20-9-99	< 0,280	< 0,147	< 0,155	< 0,064
				minimum	0,280	0,147	0,155	0,064
				maximum	0,495	0,260	0,273	0,113
				mediaan	0,352	0,185	0,194	0,080
				90 percentiel	0,471	0,248	0,260	0,107
Process water								
Petrusplaat	WBB	Finished product	W.LW.3.WBB.APE.O	20-9-99	< 0,297	< 0,156	< 0,164	< 0,068
Scheveningen	DZH	Collected after extraction	W.LW.3.DZ2.APE.O	21-9-99	< 0,381	< 0,200	< 0,210	< 0,087
Ouddorp	Delta	Collected process water	W.OW.3.OUD.APE.F	21-9-99	< 0,360	< 0,189	< 0,199	< 0,082
Drinking water								
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.3.DZ1.APE.O	21-9-99	< 0,356	< 0,187	< 0,196	< 0,081
Ouddorp	Delta	Outgoing water	W.LW.3.OUD.APE.O	21-9-99	< 0,383	< 0,201	< 0,211	< 0,087
Braakman	Delta	Outgoing water	W.LW.3.BRA.APE.O	20-9-99	< 0,335	< 0,176	< 0,185	< 0,076
Kralingen	WBE	Drinking water	W.LW.3.WB1.APE.O	22-9-99	< 0,359	< 0,189	< 0,198	< 0,082
Berenplaat	WBE	Drinking water	W.LW.3.WB2.APE.O	22-9-99	< 0,510	< 0,270	< 0,490	< 0,120
				minimum	0,297	0,156	0,164	0,068
Legend: <* = results are an indication for they are beyond the levels of certain quantification				maximum	0,510	0,270	0,490	0,120

Phase 3 September 1999 Alkylphenol ethoxylates analysis Rhine

Location	Company		Sample code	Sample date	Nonylphenol-ethoxylates	Octylphenol-ethoxylates	Nonylphenol	Octylphenol
Surface water							Concentration in µg/l	
Lobith	RIZA	German-Dutch border	A.OW.3.LOB.APE.F	4-10-99	< 0,321	< 0,169	< 0,180	< 0,073
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.3.LKN.APE.F	22-9-99	< 0,267	< 0,140	< 0,150	< 0,061
A'dam-Rijnkanaal	GWA	Inlet Weesperkarspel	W.OW.3.GW1.APE.F	22-9-99	< 0,434	< 0,228	< 0,240	< 0,099
Andijk	PWN	Inlet at IJsselmeer	W.OW.3.AND.APE.F	20-9-99	< 0,304	< 0,160	< 0,168	< 0,069
Twentekanaal	WMO	Inlet WMO	W.OW.3.WMO.APE.F	20-9-99	< 0,474	< 0,249	< 0,261	< 0,108
				minimum	0,267	0,140	0,150	0,061
				maximum	0,474	0,249	0,261	0,108
				mediaan	0,321	0,169	0,180	0,073
				90 percentiel	0,458	0,241	0,253	0,104
Process water								
Nieuwegein	WRK	Finished product	W.LW.3.LKN.APE.O	22-9-99	< 0,401	< 0,211	< 0,221	< 0,092
Lekkerkerk	WZHO	GLS PF 99	W.OW.3.WZ1.APE.F	20-9-99	< 0,325	< 0,171	< 0,180	< 0,074
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.3.WZ2.APE.F	20-9-99	< 0,281	< 0,148	< 0,155	< 0,064
Leiduin	GWA	After infitration (a.i.)	W.LW.3.GW2.APE.O	22-9-99	< 0,358	< 0,188	< 0,197	< 0,082
Drinking water								
Leiduin	GWA	Drinking water	W.LW.3.GW1.APE.O	22-9-99	< 0,316	< 0,166	< 0,174	< 0,072
Weesperkarspel	GWA	Drinking water	W.LW.3.GW3.APE.O	22-9-99	< 0,396	< 0,208	< 0,218	< 0,090
Andijk	PWN	Drinking water	W.LW.3.AND.APE.O	20-9-99	< 0,390	< 0,205	< 0,215	< 0,089
Twentekanaal	WMO	Drinking water	W.LW.3.WMO.APE.O	20-9-99	< 0,290	< 0,150	< 0,280	< 0,070
				minimum	0,281	0,148	0,155	0,064
				maximum	0,401	0,211	0,280	0,092

Legend: <* = results are an indication for they are beyond the levels of certain quantification

Phase 1 **March 1999**

General parameters in Meuse

Location	Company	Description	Sample code	Sample date	Chloride	Suspended particles	Dry residue
					mg/l	mg/l	mg/l
Surface water							
Remilly	BIWM	French-Belgian border	W.OW.1.REM.ALW.F	8-3-99	12	30	
			W.OW.1.REM.ZS.F	8-3-99		< 10	n.d.
Tailfer	BIWM	Meuse: inlet of BIWM	W.OW.1.TAI.ALW.F	8-3-99	14	30	
			W.OW.1.TAI.ZS.F	8-3-99			-
Namêche	AWW	Meuse	W.OW.1.NAM.ALW.F	8-3-99	20	70	
			W.OW.1.NAM.ZS.F	8-3-99		< 10	n.d.
Liège	AWW	Albert kanaal	W.OW.1.TAI.ALW.F	8-3-99	18	45	
			W.OW.1.TAI.ZS.F	8-3-99			-
Eijsden	RIZA	Belgian-Dutch border	W.OW.1.EYS.ALW.F	23-3-99	21		
			W.OW.1.EYS.ZS.F	23-3-99		< 10	
Belfeld	RW-DL	Meuse (NL)	W.OW.1.BEL.ALW.F	9-3-99		40	
			W.OW.1.BEL.ZS.F	9-3-99			-
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.1.KEI.ALW.F	9-3-99	42	-	
			W.OW.1.KEI.ZS.F	9-3-99			-
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.1.WBB.ALW.F	9-3-99	25	35	
			W.OW.1.WBB.ZS.F	9-3-99		< 10	n.d.
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.1.BRA.ALW.F	8-3-99	96	< 10	
			W.OW.1.BRA.ZS.F	8-3-99		< 10	n.d.
Proceswater							
Petrusplaat	WBB	Finished product	W.LW.1.WBB.ALW.O	9-3-99	36		
Scheveningen	DZH	Collected after extraction	W.LW.1.DZ2.APE.O	8-3-99	< 0.05		
Drinking water							
Scheveningen	DZH	Collected after filtration	W.LW.1.DZ1.APE.O	8-3-99	0.1		

These parameters were measured in March and June 1999
n.d. = not determined; analysis not possible

Phase 1 **March 1999** **General parameters in Rhine**

Location	Company	Description	Sample code	Sample date	Chloride	Salinity	Suspended particles	Dry residue
					mg/l	%	mg/l	mg/l
Surface water								
Karlsruhe	TZW	Rhine (D)	W.OW.1.KRL.ALW.F	8-3-99	35			
Köln	GEW	Site 1	W.OW.1.KO1.ALW.F	8-3-99	501			
			W.OW.1.KO1.ZS.F	8-3-99	63			
Köln	GEW	Site 2	W.OW.1.KO2.ALW.F	8-3-99	956			
Köln	GEW	Site 3	W.OW.1.KO3.ALW.F	8-3-99	877			
Köln	GEW	Site 4	W.OW.1.KO4.ALW.F	8-3-99	1000			
Köln	GEW	Site 5	W.OW.1.KO5.ALW.F	8-3-99	913			
Köln	GEW	Site 6	W.OW.1.KO6.ALW.F	8-3-99	677			
Lobith	RIZA	German-Dutch border	W.OW.1.LOB.ALW.F	15-3-99	55	25,40		
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.1.LKN.ALW.F	8-3-99			35	
Andijk	PWN	Inlet at IJsselmeer	W.OW.1.AND.ALW.F	8-3-99	96			
			W.OW.1.AND.ZS.F	8-3-99			< 10	n.d.
Twentekanaal	WMO	Inlet WMO	W.OW.1.WMO.ALW.F	8-3-99	-			
Proceswater								
Nieuwegein	WRK	Finished product	W.LW.1.LKN.APE.O	8-3-99	-			
Lekkerkerk	WZHO	GLS PF 99	W.OW.1.WZ1.APE.F	8-3-99				
Nieuw Lekkerland	WZHO	GPU PE 99B (De Put)	W.OW.1.WZ2.APE.F	8-3-99	140			
			W.OW.1.WZ2.ZS.F	8-3-99			10	
Leiduin	GWA	After infitration (a.i.)	W.LW.1.GW2.APE.O	8-3-99	101.8			
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.1.GW3.APE.O	8-3-99	101.7			
Leiduin	GWA	After active carbon filtration (a.c.)	W.LW.1.GW4.APE.O	8-3-99	107.6			
Drinkwater								
Leiduin	GWA	Drinking water	W.LW.1.GW1.APE.O	8-3-99	108.4			
Andijk	PWN	Drinking water	W.LW.1.AND.APE.O	8-3-99	125		< 10	
WMO	WMO	Drinking water	W.LW.1.WMO.APE.O	8-3-99	-			

These parameters were measured in March and June 1999

n.d. = not determined; analysis not possible

Phase 2 June 1999 General parameters in Meuse

Sample site	Company	Description	Sample code	Sample date		particles	
					mg/l	mg/l	mg/l
Surface water							
Remilly	BIWM	Frans-Belgische grens	W.OW.2.REM.ZS.F	28-6-99		15	<
			W.OW.2.REM.ALW.F	28-6-99	16		
Tailfer	BIWM	Maas: inlaat BIWM	W.OW.2.TAI.ZS.F	28-6-99		30	<
			W.OW.2.TAI.ALW.F	28-6-99	19		
Namêche	AWW	Maas	W.OW.2.NAM.ZS.F	28-6-99		40	<
			W.OW.2.NAM.ALW.F	28-6-99	32		
Liège	AWW	Albertkanaal	W.OW.2.LUI.ZF.F	28-6-99		15	<
			W.OW.2.LUI.ALW.F	28-6-99	45		
Eijsden	RIZA	Belgisch-Nederlandse grens	A.OW.2.EYS.ZS.F	13-7-99		-	-
			A.OW.2.EYS.ALW.F	13-7-99	-		
Heel	WML	Lateraalkanaal at production facility Heel	W.OW.2.WPH.ZS.F	30-6-99		< 10	<
			W.OW.2.WPH.ALW.F	30-6-99	41		
Roosteren	WML	Grensmaas at production facility Roosteren	W.OW.2.WPR.ZS.F	30-6-99		20	<
			W.OW.2.WPR.ALW.F	30-6-99	43		
Belfeld	RW-DL	Meuse (NL)	W.OW.2.BEL.ZS.F	29-6-99		< 10	<
			W.OW.2.BEL.ALW.F	29-6-99	44		
Keizersveer	RW-DZH	Meuse: reference site of WBB and DZH	W.OW.2.KEI.ZS.F	29-6-99		15	<
			W.OW.2.KEI.ALW.F	29-6-99	48		
Biesbosch	WBB	Gat van de Kerksloot: inlet of WBB	W.OW.2.WBB.ZS.F	28-6-99		15	<
			W.OW.2.WBB.ALW.F	28-6-99	47		
Brakel	DZH	Inlet at Afgedamde Maas	W.OW.2.BRA.ZS.F	28-6-99		< 10	<
			W.OW.2.BRA.ALW.F	28-6-99	43		

These parameters were measured in March and June 1999

Phase 2 **June 1999** **General parameters in Meuse**

Continued

Sample site	Company	Description	Sample code	Sample date	Chloride	Suspended particles	Dry residue
					mg/l	mg/l	mg/l
Process water							
Petrusplaat	WBB	Finished product	W.LW.2.WBB.ALW.O	28-6-99	33		
Scheveningen	DZH	Collected after extraction	W.LW.2.DZ2.ALW.O	29-6-99	49		
Ouddorp	Delta	Collected process water after infiltration	W.OW.2.OUD.ALW.F	29-6-99	72		
			W.OW.2.OUD.ZS.F	29-6-99		< 10	<
Drinking water							
Scheveningen	DZH	Collected after filtration; drinking water	W.LW.2.DZ1.ALW.O	29-6-99	50		
Ouddorp	Delta	Drinking water	W.LW.2.OUD.ALW.O	29-6-99	71		
Braakman	Delta	Drinking water	W.LW.2.BRA.ALW.O	29-6-99	33		
Kralingen	WBE	Drinking water	W.LW.2.WB1.ALW.O	30-6-99	42		
Berenplaat	WBE	Drinking water	W.LW.2.WB2.ALW.O	30-6-99	43		

These parameters were measured in March and June 1999

Phase 2 June 1999 General parameters in Rhine

Sample site	Company	Description	Sample code	Sample date	Chloride mg/l	Suspended particles mg/l	Dry residue mg/l
Surface water							
Lobith	RIZA	German-Dutch border	A.OW.2.LOB.ALW.F	5-7-99	81		
			A.OW.2.LOB.ZS.F	5-7-99		25	<
Nieuwegein	WRK	Lekkanaal: inlet	W.OW.2.LKN.ALW.F	30-6-99	70		
			W.OW.2.LKN.ZS.F	30-6-99		60	<
Andijk	PWN	Inlet at IJsselmeer	W.OW.2.AND.ALW.F	28-6-99	77		
			W.OW.2.AND.ALW.F	28-6-99		< 10	<
Twentekanaal	WMO	Inlet WMO	W.OW.2.WMO.ALW.F	28-6-99	76		
			W.OW.2.WMO.ZS.F	28-6-99		10	<
Process water							
Nieuwegein	WRK	Finished product	W.LW.2.LKN.ALW.O	30-6-99	71		
			W.LW.2.LKN.ZS.O	30-6-99			
Lekkerkerk	WZHO	GLS PF 99	W.OW.2.WZ1.ALW.F	28-6-99	125		
			W.OW.2.WZ1.ZS.F	28-6-99		< 10	<
Nieuw Lekkerlan	WZHO	GPU PE 99B (De Put)	W.OW.2.WZ2.ALW.F	28-6-99	120		
			W.OW.2.WZ2.ZS.F	28-6-99		< 10	<
Leiduin	GWA	After infitration (a.i.)	W.LW.2.GW2.ALW.O	29-6-99	96		
Leiduin	GWA	After ozone treatment (a.o.)	W.LW.2.GW3.ALW.O	29-6-99	96		
Drinking water							
Leiduin	GWA	Drinking water	W.LW.2.GW1.ALW.O	29-6-99	93		
Weesperkarspel	GWA	Drinking water	W.LW.2.GW4.ALW.O	29-6-99	78		
Andijk	PWN	Drinking water	W.LW.2.AND.ALW.O	28-6-99	100		
Twentekanaal	WMO	Drinking water	W.LW.2.WMO.ALW.O	28-6-99	56		

These parameters were measured in March and June 1999