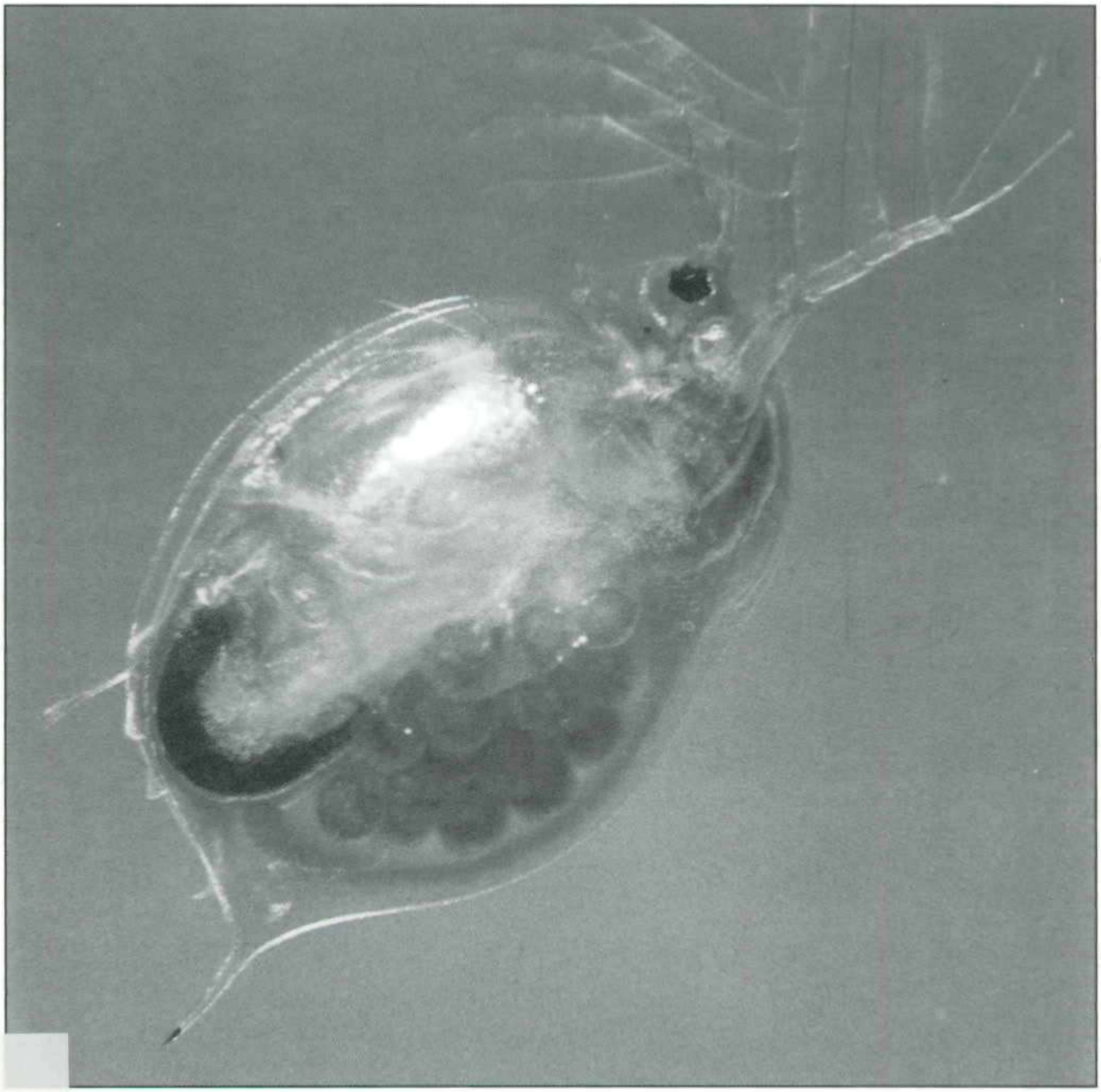
Monitoring concentrations of microcontaminants and response of waterfleas and fish in the Rhine Delta

Hendriks A.J. (ed.)

Report 94.027



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RIZA

Institute for Inland Water Management and Waste Water Treatment

P.O. Box 17, 8200 AA Lelystad, the Netherlands

tel. NL-320070411 fax. NL-320049218

Monitoring concentrations of microcontaminants and response of waterfleas and fish in the Rhine delta

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author A.J. Hendriks (ed.)

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SUMMARY

This report describes the results of several studies carried out in the framework of the international "Rhine Action Program" and the Dutch cooperation "Ecological Rehabilitation of the Rivers Rhine and Meuse" (Section 1.1).

Though water and sediment concentrations of many compounds have decreased, levels for some of them -especially cadmium, copper, mercury, zinc, hexachlorobenzene, polycyclic aromatic hydrocarbons and chlorobiphenyls are above Dutch quality standards (Chapter 2.). These standards probably reflect a minimum for achieving the goals set by the Rhine Action Program.

Concentrations of several priority contaminants in aquatic organisms have decreased as well (Chapter 3.). Yet, the decline tends to level off for some compounds, whereas residues of other pollutants did not decrease at all. Levels considered safe for consumption by humans or animals may vary by several orders of magnitude, depending on the data and methods used. However, the lowest critical value for anyone compound is within a factor 10 of 100 $\mu g \cdot kg^{-1}$ wet weight, a level that is repeatedly exceeded for cadmium, mercury and PCBs.

Whereas waterfleas died immediately after exposure to Rhine water in the seventies, no acute response was observed in recent samples (Chapter 4.). Yet, chronic effects were noted after 5-10 fold concentration of hydrophilic to moderately lipophilic organic compounds in Rhine water samples. It indicates that more sensitive species may still be affected. Moreover, less than 15% of the response observed could be attributed to the pollutants identified by GC-MS (158 compounds identified). The rest is likely to be caused by unknown pollutants, stressing the need for toxicity assays in surface water and effluent monitoring.

To monitor peak loads of miscellaneous contaminants, biological early warning systems were installed at Lobith (Rhine) and Eijsden (Meuse). The number of alerts produced by the fish system at Lobith has decreased to a low level, similar to that of up- and downstream systems (DRC-Arbeitsgruppe "Dynamische Wirkungstests Rhein", RIWA/RIZA Working Group "Bioalarm"). Recent technical improvements as well as the implementation of the more sensitive waterflea system are expected to increase the number of biological alerts produced (Chapter 5.).

Currently concentration monitoring efforts are extended with less well-known compounds. The interpretation of concentration- and response monitoring will be refined to separate sections of the Rhine. Moreover, the significance of the results for other, less well-known species is investigated in modelling projects (Section 1.2.).

SAMENVATTING

Dit rapport beschrijft de resultaten van enkele studies die zijn uitgevoerd in het kader van het internationale "Rijn Actie Programma" en de Nederlandse samenwerking "Ecologisch Herstel van de rivieren Rijn en Maas" (paragraaf 1.1).

Hoewel water en sediment concentraties van veel stoffen zijn afgenomen, bevinden de niveaus van sommige stoffen -in het bijzonder cadmium, koper, kwik, zink, hexachloorbenzeen, polycyclische aromatische koolwaterstoffen en chloorbifenylen- zich boven die van de normen (hoofdstuk 2.). Deze normen weerspiegelen waarschijnlijk een niveau dat minimaal nodig is om de doelstellingen van het Rijn Actie Programma te verwezenlijken.

Concentraties van een aantal prioritaire verbindingen in aquatische organismen zijn ook afgenomen (hoofdstuk 3.). Echter, de afname lijkt af te vlakken voor een aantal stoffen, terwijl concentraties van andere microverontreinigingen gelijk zijn gebleven. Concentraties in organismen die veilig geacht worden voor consumptie door mensen en dieren kunnen enkele ordes van grootte verschillen, afhankelijk van de gegevens en de methodes die gebruikt zijn. Echter, de laagste kritische niveaus voor het merendeel van de gemeten stoffen bevindt zich binnen een factor 10 van de 100 $\mu g \cdot kg^{-1}$ natgewicht. Dit niveau wordt regelmatig overschreden voor cadmium, kwik en PCBs.

In de zeventiger jaren stierven watervlooien onmiddellijk wanneer ze werden blootgesteld aan Rijn water maar in recente monsters werd geen acute sterfte waargenomen (hoofdstuk 4.). Echter, in sommige monsters traden chronische effecten op na 5 tot 10-voudige concentratie van de hydrofiele tot matig lipofiele organische microverontreinigingen. Dit suggereert dat gevoeliger soorten waarschijnlijk nog steeds beïnvloed worden door deze verbindingen. Bovendien, minder dan 15% van de waargenomen giftigheid kon verklaard worden met de verbindingen die werden geïdentificeerd met GC-MS analyse (158 stoffen werden geïdentificeerd). Het resterende deel werd waarschijnlijk veroorzaakt door onbekende verbindingen. Dit benadrukt het belang van giftigheidstoetsen in oppervlakte- en afvalwater metingen.

Om piekbelastingen van diverse stoffen te registreren, zijn biologische bewakingssystemen geïnstalleerd bij Lobith (Rijn) en Eijsden (Maas). Het aantal alarmmeldingen van het visbewakingssysteem bij Lobith is afgenomen tot een laag niveau, conform ervaringen in boven- en benedenstroomse delen van de Rijn (DRC-Werkgroep "Dynamische Wirkungstests Rhein", RIWZ/RIZA Werkgroep "Bioalarm"). Door recente technische verbeteringen en installatie van een gevoeliger watervlosysteem zal naar verwachting het aantal biologische alarmmeldingen toenemen (hoofdstuk 5.).

Momenteel worden de concentratiemetingen uitgebreid met minder bekende verbindingen. De interpretatie van concentratie en -responsmetingen wordt verfijnd tot specifieke delen van de rivier. Bovendien wordt het belang van de resultaten voor andere, minder bekende soorten onderzocht in model-achtige studies (paragraaf 1.2).

CONTENTS

1. INTRODUCTION

1.1. Objectives

This report is a compilation of recently published papers on microcontaminants in the Rhine-delta. The results were obtained in studies carried out within the framework of the international "Rhine Action Program" (IRC 1988, 1992, 1993) and the Dutch cooperation "Ecological Rehabilitation of the Rivers Rhine and Meuse" (Admiraal et al. 1992, Van Dijk and Marteijn 1993).

Since the journals concerned may not be readily available to policy-making and management departments it was considered appropriate to combine them into one report here. Moreover, it allows for a brief outline of past, forthcoming and future work in this area. To improve readability, some minor adjustments were made to the original manuscripts.

Chapter 2. and 3. give an overview of concentrations of several priority contaminants in water, sediment and aquatic organisms. Chapter 4. describes the experiences with response of waterfleas to average loads of hydrophilic to moderately lipophilic contaminants. Finally, chapter 5. focuses on the response of waterfleas and fish to peak loads of miscellaneous contaminants in biological early warning systems.

1.2. Prospects

Concentration monitoring programs, similar to those reported in chapter 2. and 3. have been executed internationally (IRC 1993). Future efforts in the Netherlands are directed towards more refinement in terms of scale and the number of compounds measured (Van Steenwijk et al. 1992, Maas 1993, Hendriks and Pieters in prep., Eys and Hendriks in prep.). The levels measured will be compared to quality standards for which (inter-)national consensus has been reached (IRC 1992, Ministry of Housing, Physical Planning and Environment 1993).

Monitoring by static assays, as described in chapter 4. has been and will be intensified in terms of frequency and sample locations (Maas 1993, De Zwart and Folkerts 1990, Eys and Hendriks 1995 in prep.) In addition, some of the assumptions made in chapter 4. will be tested for their validity (Maas and Hendriks, in prep.).

In conformity with international recommendations (Schmitz 1994), there is an intention to extent biological early warning, as reported in chapter 5., with algae and bacteria systems in the years to come. A feasibility-study for the application of biological early warning systems at effluents has been completed recently (Okkermans et al. 1993).

Monitoring by chemical analysis and laboratory assays gives an indication of the risk for the aquatic species tested (and used to derive quality standards). In addition to this, modelling helps to identify specific (groups of) compounds and species that are most troublesome. Results from species specific estimations, backed by calibration and validation efforts, including those in floodplains have been and will be reported separately (e.g. Van Brummelen 1990, Dogger et al. 1992, Balk et al. 1993, Hendriks 1994a, 1994b, 1994c, Hendriks et al. 1994, Reinhold et al. 1994, De Wit et al. 1991).

Acknowledgements

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2. MONITORING CONCENTRATIONS OF MICROCONTAMINANTS IN SEDIMENT AND WATER IN THE RHINE DELTA: A COMPARISON WITH REFERENCE VALUES

A. JAN HENDRIKS

Institute for Inland Water Management and Waste Water Treatment,
P.O. Box 17, 8200 AA Lelystad, The Netherlands

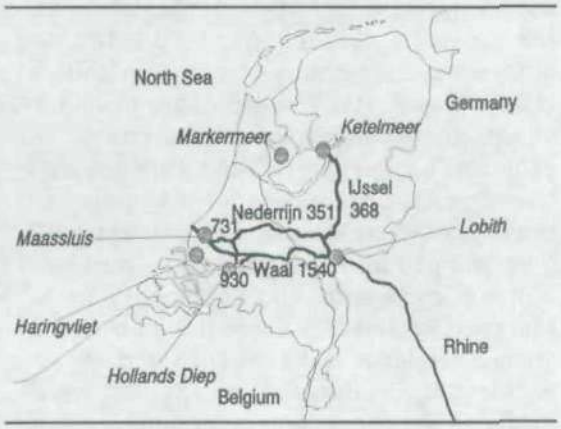
Abstract

Sediment and suspended solid concentrations of many microcontaminants are above Dutch quality standards. Especially levels of cadmium, copper, mercury, zinc, hexachlorobenzene, polycyclic aromatic hydrocarbons and chlorobiphenyls are of concern. Some chlorobiocides, such as drins, have become less significant but others, such as α-endosulfan and hexachlorocyclohexanes are still important. The quality standards are believed to protect most species and may be considered a minimum for achievement of the goals set by the "Rhine Action Program".

The sharp decline of water concentrations by some heavy metals seems to slow down close to quality standards. Reviewing major pollution incidents from the period 1988-1991 indicates that half of these probably do not reach lethal levels. The impact of other incidents remains unknown because of a lack of information.

2.1. Introduction

Figure 1. Major river and lakes of the Rhine delta and sample locations (italics) with river discharges $m^3 \cdot s^{-1}$.



The Dutch Rhine delta consists of several interconnected rivers and lakes, as shown in Figure 1. It serves as a source for industrial, drinking, fishing and irrigation water. In particular, surface water in lower areas of The Netherlands may contain up to 50% or more Rhine water.

The rivers and lakes are also used as media for shipping and recreation and as sinks for municipal and industrial effluents and agricultural runoff. The concern for the consequences of this exploitation has brought about the "Rhine Action Program". One of its main objectives is to reduce pollution and to restore destroyed habitats of indigenous species (IRC 1987).

As a first step, the countries concerned have agreed to reduce regular immissions of priority micropollutants (Vrijhof 1984) by 50% during the period 1985 to 1995 (IRC 1987). Unfortunately, even realization of higher percentages is no guarantee for achievement of the goal set.

Though priority pollutants are probably most hazardous, other compounds among the thousands estimated to be present may be important as well. Also, contaminants that are no longer tolerated might be substituted by other toxic compounds, such as possible replacement of chlorobiphenyls (Aroclor) by chlorobenzyltoluenes (Ugilec). Reduction does not necessarily lead to an immediate proportional decline in concentrations. Concentrations of

compounds that occur naturally will decrease less than proportionally and it may take up many years to reach new steady states for persistent chemicals in sediments. The average degree of contamination achieved after reduction may still be too high for indigenous species. Current estimations of "safe levels" are mainly derived from experiments in which one observes a limited set of standard species and effects. Besides, large temporal (episodes) and spatial (mixing zones) variations may still prevent permanent and widespread residence of indigenous species.

These potential bottle-necks are studied in several projects. Concentrations in water, sediment (this paper), in organisms (Hendriks and Pieters 1993) as well as damage observed in assays (Hendriks et al. 1994, Hendriks and Stouten 1993) have been studied. This paper focuses on monitoring micropollutants in sediment and water in the Dutch Rhine delta. The data presented cover the period 1985-1990. The locations sampled represent most of the Rhine delta. Central, western and northern branches are represented by dotted, hatched and cross-hatched bars in the figures of this paper.

2.2. Methods

2.2.1. Water concentrations

Monitoring programs of the Institute for Inland Water management and Waste Water Treatment provided most of the concentrations in water (Heymen 1990). The samples are taken from the river branches at the monitoring stations of Lobith and Maassluis, as illustrated in Concentrations of most compounds were registered monthly at each location during 1985-1989. Averages cover at least 6 individual data per location. The coefficient of variation σ/μ exceeded 2 for some concentrations of dichloronitrobenzenes, dichlorobenzenes and some chlorobiocides. The average coefficient was 0.7.

Long-term differences will be presented by comparing annual averages of metal concentrations in 1980, 1985 and 1990. Since 1988, lipophilic organic microcontaminants were no longer monitored in total water but in suspended solids samples (Venema 1991) so that no trends can be given. Short-term variation due to episodes es since 1988 will be reported (Schäfer and Breukel 1990).

Spatial variation in water concentrations along the river branches turned out to be small on a national scale. We will therefore confine ourselves to data from Lobith and Maassluis. At Maassluis pollution levels may diluted by the inflow of sea water but samples are taken at moments of minimal dilution.

The importance of local variation along the river branches may be coarsely estimated by the ratio of input [$\mu g \cdot s^{-1}$] (RIZA 1990) and river discharge [$l \cdot s^{-1}$] given in Figure 1. under the assumption of immediate and complete mixing. It will be reported as a percentage of the concentration measured at the upstream location of Lobith. This simple estimation does not hold for non-conservative compounds and extra dilution by tidal movement.

2.2.2. Sediment concentrations

Concentrations in sediments have been investigated by different local authorities during the period of 1985-1989. Some of the variability reported may be due to differences in methods of sampling and chemical analysis. Therefore levels will be interpreted as indicative values. The sediment samples have been arranged into five regions. Scarce data for the upper branches IJssel, Waal and Nederrijn have been merged into one group. The northern region is represented by Ketelmeer and Markermeer and the western area by the successive lakes Hollands Diep and Haringvliet. On the average, 80 data were available per region and compound.

The coefficient of variation σ/μ lies within a range that is similar to the variation of concentrations in water. The average coefficient was 1.1. It could mean that the extra number of samples per sediment location that contribute to the average conceals larger spatial variation. Coefficients of variation higher than 2 were

calculated for chlorobiphenyls concentrations in Haringvliet.

As an indication of long-term variation, suspended solid concentrations measured at Lobith in 1990 are added to the sediment graphs. Sediment concentrations are reported in dry weight, as $\mu g \cdot kg^{-1}$ dry weight, after normalization to 5% organic carbon and 25% lutum contents, according to a common procedure (e.g. Venema 1991). For suspended solids these percentages are 10% and 40% respectively.

2.2.3. Reference concentrations

The concentrations in water and sediment will be compared to current Dutch quality standards (Stortelder et al. 1991, Van der Gaag 1991). These standard generally reflect the highest concentration in water or in food without chronic damage to laboratory species, corrected for combined exposure by concentration addition. Concentrations in water, sediment and organisms were translated under the assumption of equilibrium partitioning (Van der Kooij et al. 1991). Estimated natural background concentrations for heavy metals and polycyclic aromatic hydrocarbons (Van der Meent et al. 1990) may also serve as a guide-line.

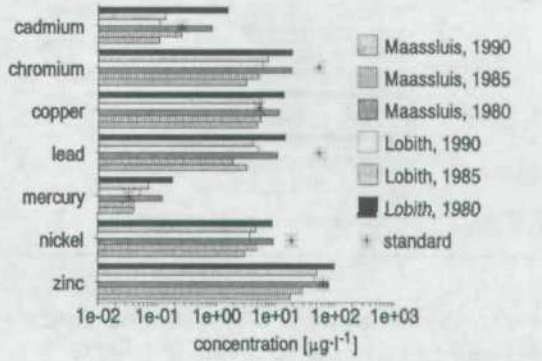
No standard has been derived for most of the irregularly released compounds. To obtain at least some idea of their impact, we will refer to acute median lethal concentrations that are extracted from databases (Pilli et al. 1989, BKH 1990).

2.3. Results and discussion

2.3.1. Heavy metals

Concentrations of heavy metals in water and sediment are scattered around the Dutch quality standards, while natural background levels are one order of magnitude lower. Average chromium and lead concentrations are about 5 times lower than these water quality standards but contamination by other metals is worse.

Figure 2. Heavy metal concentrations in water volume of the Rhine delta during 1980, 1985 and 1990, compared to standards concentrations.



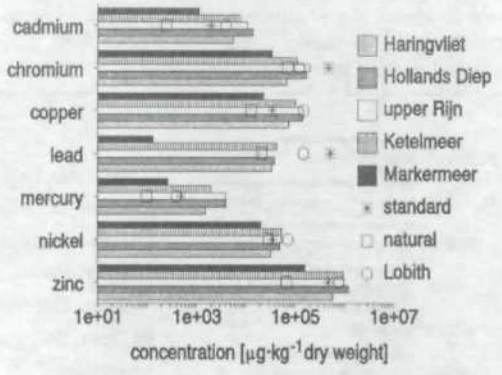
The decline in water concentrations that started in the seventies continues in the eighties as illustrated in Figure 2. Comparing annual averages of 1980, 1985 and 1990 shows that the trend tends to level off.

Concentrations at Maassluis are lower than at Lobith with the exception of cadmium in 1985. Comparing industrial immissions (RIZA 1990) and river discharges to the upstream concentration suggests that this may be attributed to input in the Rotterdam harbor area. Similar estimations indicate that relatively important inputs of zinc are found along the IJssel. These immissions may add about 10% to the concentration measured at Lobith.

Figure 3. summarizes heavy metal contamination of sediments. Comparing suspended solid concentrations of 1990 at Lobith to sediment contamination during the period 1985-1989, one may conclude that heavy metal pollution of sediments has not or only slightly improved. For each heavy metal, the highest concentrations are found in upper Rijn (IJssel, Nederrijn, Waal), Nieuwe Merwede and/or Hollands Diep. Markermeer is clearly less polluted, though cadmium and mercury concentrations are several times higher than natural background levels. Increased concentrations in Hollands Diep might (partially) be explained by input from the Maas.

Flood plains have not been investigated as extensively as wet sediments. Heavy

Figure 3. Normalized heavy metal concentrations in suspended (Lobith) and sedimented (other locations) dry mass of the Rhine delta during the period of 1985-1990, compared to standard and natural concentrations.



metal concentrations in suspended solids proved to be equal to levels in freshly deposited upper layers in the major bed (Japenga et al. 1990). In the seventies, concentrations in some plains outside the dike were on the same level as those of Figure 3. This proved to be up to 60 times higher than a reference location behind the dike (Van de Ven et al. 1977).

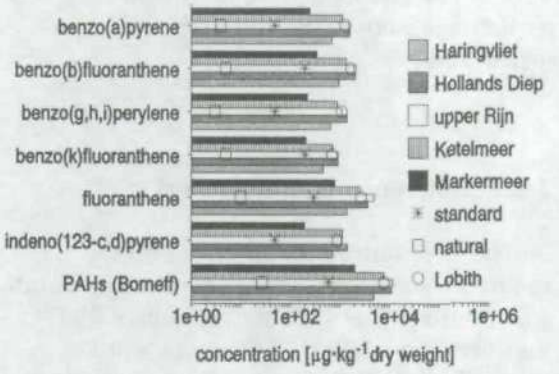
2.3.2. Monocyclic aromatic hydrocarbons

Monocyclic aromatic hydrocarbons, such as (ethyl)benzene, styrene, toluene and xylene, are no longer regularly monitored in water. Current levels of ethylbenzene and benzene are about 50 times lower than quality standards (not shown). Large industrial immissions of benzene of 1,000 to 10,000 $kg \cdot y^{-1}$ (RIZA 1990) may induce higher concentrations near point sources but concentrations will decrease rapidly due to volatilization.

2.3.3. Polycyclic aromatic hydrocarbons

Samples collected in both water and sediment have yielded averages and maxima of polycyclic aromatic hydrocarbons concentrations that are well above standard and natural background values. Non-reported data indicated a decline of Borneff polycyclic aromatic hydrocarbons concentrations in water. All known industrial point immissions into the

Figure 4. Normalized Borneff polycyclic aromatic hydrocarbons concentrations in suspended (Lobith) and sedimented (other locations) dry mass during the period of 1985-1990, compared to standard and natural concentrations.



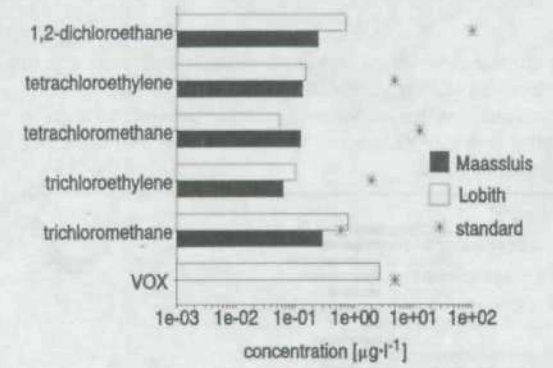
Rhine are less than 5 $kg \cdot y^{-1}$ (RIZA 1990) except one input in Rotterdam, which may add several percentages to the concentration measured at Lobith. Diffusive input of these hydrocarbons into Dutch water exceeds major industrial point discharges by more than a factor 10 (RIZA 1990).

Figure 4. shows that sediment quality standards are exceeded in all regions. Nieuwe Merwede and upper Rijn are highly polluted with all kinds of compounds, but concentrations of some polycyclic aromatic hydrocarbons are especially high in comparison to other regions. This may indicate the importance of small local sources, because they are probably more than proportionally represented in samples from rivers compared with lakes.

2.3.4. Chloroaliphatic hydrocarbons

Contamination by traditional volatile chloroaliphatic hydrocarbons has dropped sharply since 1980. Figure 5. gives current levels. In 1989, concentrations for most compounds were below detection limits. In Figure 9., one can see that reported episodes were also well below median lethal concentrations extracted from databases

Figure 5. Chloroaliphatic hydrocarbon concentrations in water volume of the Rhine delta during the period of 1985-1990, compared to standard concentrations.



(BKH 1990, Pilli et al. 1989).

Around Maassluis some local problems might exist because of large industrial immissions of 1,2-dichloroethane and tetrachloroethylene.

2.3.5. Monocyclic chloroaromatic hydrocarbons

The regularly monitored monocyclic substituted aromatic hydrocarbons consists of chlorobenzenes, with or without hydroxy- or nitro-groups.

Figure 6. Monocyclic substituted aromatic hydrocarbon in water volume of the Rhine delta during the period of 1985-1990, compared to standard concentrations.

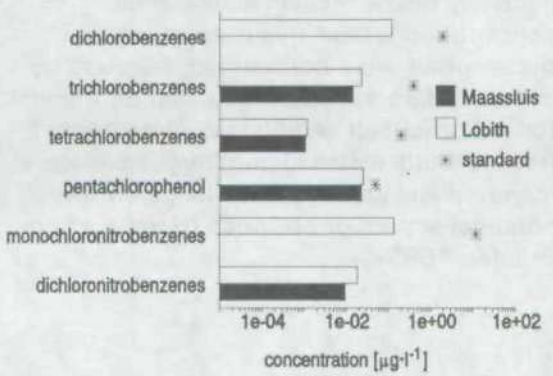


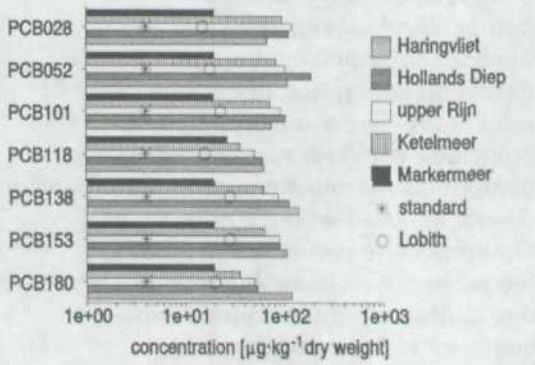
Figure 6. and Figure 8. demonstrate that -in general- problematical compounds of this category are pentachlorophenol and especially hexachlorobenzene. Comparing sediment concentrations to recent suspended solid concentrations in Lobith in Figure 6. shows that contamination is decreasing.

Substituted benzenes are often reported as episodes but Figure 9. demonstrates that median lethal concentrations are usually higher than 500 $\mu g \cdot l^{-1}$ for these compounds. Two major industrial point immissions of hexachlorobenzene are located near Rotterdam. Though their loads were less than 10 $kg \cdot y^{-1}$ (RIZA 1990) in 1985 this input may increase the river concentration by 25%.

2.3.6. Chlorobiphenyls and -dibenzodioxins

Routinely monitored chlorobiphenyls include the congeners 28, 52, 101, 118, 138, 153 and 180. Spatial variation in water is small as the largest industrial point immissions of 0.1 $kg \cdot y^{-1}$ (RIZA 1990) located along the upper branches are insignificant compared to communal and diffusive input.

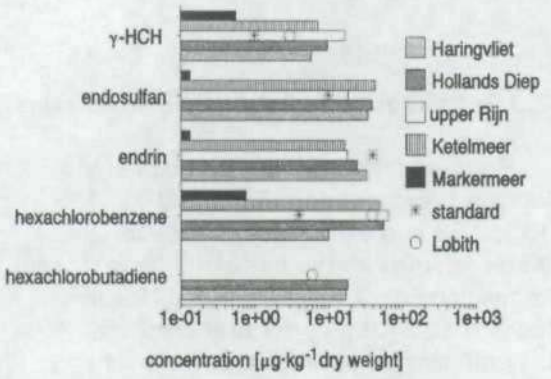
Figure 7. Normalized chlorobiphenyl concentrations in suspended (Lobith) and sedimented (other locations) dry mass of the Rhine delta during the period of 1985-1990, compared to standard concentrations.



In sediment, concentrations are also well above quality standards, even in relatively unspoilt lakes, such as Markermeer and IJsselmeer. Related compounds, such as chlorodibenzodioxins and -furanes have incidentally been measured. Concentrations of total chlorodibenzodioxins and -furans at normalized suspended solids ranged between 0.01 and 0.1 $\mu g \cdot kg^{-1}$ TCDD-equivalents (Turkstra and Pols 1989). Up to 0.2 and 5 $\mu g \cdot kg^{-1}$ TCDD-equivalents were detected in river (Evers et al. 1988) and harbor (Turkstra and Pols 1989) sediments respectively.

2.3.7. Chlorobiocides

Figure 8. Normalized chlorobiocides and -hexachlorobenzene concentrations in suspended (Lobith) and sedimented (other locations) dry mass of the Rhine delta during the period of 1985-1990, compared to standard concentrations.

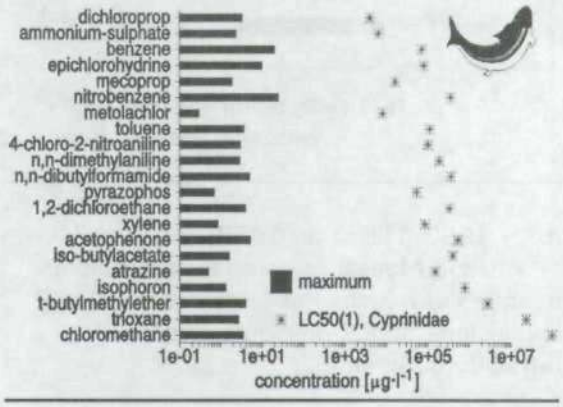


Pollution by some traditional accumulating chlorobiocides at suspended solids have dropped substantially. Average suspended solid concentrations of α-endosulphan, several drins, heptachloro(epoxid) and hexachlorobutadiene are now close to detection limits, and about one order of magnitude below quality standards. This coincides with low residues of these compounds in sediment-dwelling organisms (Hendriks and Pieters 1993). From Figure 8., one can conclude that γ-hexachlorocyclohexane (lindane) and α-endosulfan are most troublesome in sediments. Concentrations of dichlorodiphenyltrichloroethane (DDT) and its derivatives (DDD, DDE) are often below detection limits in sediments. Nevertheless, the average suspended solid concentration was 24 $\mu g \cdot kg^{-1}$ at Lobith in 1990, which is above the quality standard.

In general, chlorobiocide load comes from communal and diffusive immissions and from abroad (RIZA 1990, IRC 1989). In The Netherlands, industrial point input is confined to input of drins near Rotterdam.

2.3.8. Other compounds

Figure 9. Maximum concentrations in water volume at episodes during 1989-1991, compared to the estimated lethal concentrations for Cyprinidae fish after one day exposure (Pilli et al. 1989, Hendriks and Stouten 1993).



Some compounds, not mentioned so far, are monitored less intensively. Maximum concentrations of the nitrogenbiocides simazine and atrazine varied around quality standards and detection limits of about 0.1 $\mu g \cdot l^{-1}$ in 1989. Phosphorbiocides, monitored as a choline-esterasis inhibition sum, have stayed just above the quality standard of 0.5 $\mu g \cdot l^{-1}$ for many years.

Figure 9. shows the maximum concentrations reported of about half the episodes during 1988-1991. These are generally below median acute lethal concentrations. But this is no guarantee that species were not harmed, because no or insufficient toxicity data could be found for the other half. Additional information from recently installed continuous biological monitors will improve knowledge on the potential impact of episodes (Hendriks and Stouten 1993).

2.4. Conclusions

Sediment and suspended solid concentrations for many compounds are still well above Dutch quality standards. Especially cadmium, copper, mercury, zinc, hexachlorobenzene, polycyclic aromatic hydrocarbons, and chlorobiphenyls are problematical. Some chlorobiocides, such as drins, have become less significant, but

others, such as α-endosulfan and hexachlorocyclohexanes are still important. Careful interpretation is required because concentrations below detection limits are no guarantee that compounds are of no concern, as illustrated by DDT and its derivatives. The quality standards are believed to protect most species and may be considered a minimum for achievement of the goals set by the "Rhine Action Program".

The sharp decline of water contamination by some heavy metals seems to stagnate just above or below quality standards. Reviewing episodes from the period 1988-1991 indicates that half of the increased concentrations probably do not reach lethal levels. The impact of other episodes remains unknown because of a lack of information. In particular, rough estimations suggested that large immissions may increase the concentrations measured at Lobith by tens of percentages. Also, contamination of flood plains may be comparable to that of wet sediments.

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3. MONITORING CONCENTRATIONS OF MICROCONTAMINANTS IN AQUATIC ORGANISMS IN THE RHINE DELTA: A COMPARISON WITH REFERENCE VALUES

A. JAN HENDRIKS[1] AND HENK PIETERS[2]

[1]Institute for Inland Water Management and Waste Water Treatment, P.O. Box 17, 8200 AA Lelystad[1]
[2]Netherlands Institute for Fishery Research, P.O. Box 68, 1970 AB IJmuiden[2],
The Netherlands

Abstract

In this study, we measured residues of accumulating microcontaminants in seven aquatic species at seven locations in the Dutch Rhine delta and collected additional data from other papers. Comparison with preliminary quality standards suggests that a certain impact of micropollutants on biota of the aquatic community cannot be excluded. Concentrations of some traditional micropollutants, like chlorobiphenyls, mercury, and DDT tend to increase slightly in a downstream direction with exception of the last section. Residues of heavy metals are higher in invertebrates than in fish. On average, accumulation of organic compounds in invertebrate fat is half the level in fish fat. Residues that are somewhat higher than reflected by these ratios have been observed for chlorobiphenyls in fish and for mercury in pike-perch, Stizostedion lucioperca. Residues in livers of cormorants, Phalacrocorax carbo, from Ketelmeer are one order of magnitude higher.

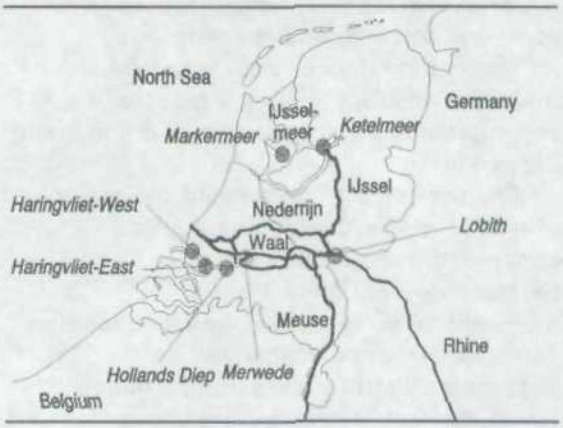
Residues of many traditional compounds have decreased substantially in during the last decade. Comparison with previous studies shows that But recently the decline is suspected to level off. Residues of some other compounds have not declined. This trend is most striking for PCB153 and PCB180 in eel, Anguilla anguilla.

3.1. Introduction

The Dutch Rhine delta consists of several interconnected rivers and lakes, outlined in Figure 10. It serves as a source for industrial, drinking, fishing and irrigation water. In particular, surface water in lower areas of The Netherlands may contain up to 50% or more Rhine water. The rivers and lakes are also used as media for shipping and recreation and as sinks for municipal and industrial effluents and agricultural runoff. The concern for the consequences of this exploitation has brought about the "Rhine Action Program". One of its main objectives is to reduce pollution and to restore destroyed habitats of indigenous species (IRC 1987).

As a first step, the countries concerned have
agreed to reduce regular immissions of priority micropollutants by 50% during the period of 1985 to 1995 (IRC 1987).

Figure 10. Major river and lakes from the Rhine delta and sample locations (italics).



Unfortunately, even the realization of higher percentages is no guarantee for achievement of the goal set.

Though priority pollutants are probably the most hazardous, other compounds among the thousands estimated to be present may be important too. Also, contaminants that are no longer tolerated might be substituted by other toxic compounds, such as the possible replacement of chlorobiphenyls (Aroclor) with chlorobenzyltoluenes (Ugilec). Reduction does not necessarily lead to an immediate proportional decline in concentrations. Concentrations of compounds that occur naturally will decrease less than proportionally. and it may take up many years to reach new steady states for persistent chemicals in sediments. The average degree of contamination achieved after reduction may still be too high for indigenous species. Current estimations of "safe levels" are mainly derived from experiments in which one observes a limited set of standard species and effects. Besides, large temporal (episodes) and spatial (mixing zones) variations may still prevent permanent and widespread residence of indigenous species.

These potential bottle-necks are studied in several projects. Concentrations in water, sediment (Hendriks 1993) and organisms (this study) as well as damage observed in assays (Hendriks et al. 1994, Hendriks and Stouten 1993) have been studied. This paper focuses on monitoring micropollutants in aquatic organisms. We will primarily confine ourselves to a comparison between residues in organisms, reference values and concentrations in sediment and suspended solids.

The compounds chosen stand for traditional micropollutants suspected to accumulate in organisms (e.g. Biddinger and Gloss 1984).

The taxa selected represent the major trophic levels of the (semi-)aquatic community in the Rhine delta, as characterized in Figure 10. As representatives of zoobenthos that feed on phytoplankton and suspended or sedimented detritus we sampled midge larvae, Chironomidae, and mussels, Bivalvia. If no zebra mussels, Dreissena polymorpha, were found, another clam, Anodonta cygnea, was sampled instead. Sediment-dwelling Oligochaeta could not be collected in quantities large enough for chemical analyses. The zooplankton collected consisted mainly of waterfleas, Cladocera. In Markermeer we could not collect enough of this genus and took scuds, Gammarus, known to thrive near stony shores.

Table 1. Niches of several taxa that thrive in the Rhine delta. Groups in bold type are sampled in this study, others have been studied in other projects mentioned in this paper.

compartment	water	wet sediment
	detritus	
plants	Schizophyta, Tracheophyta	
arthropods, molluscs	**Cladocera**	Oligochaeta
	Bivalvia	**Chironomidae**
fish, amphibians, birds, mammals	**O. eperlanus**	Riparia Aythya Chiroptera
	R. rutilus	**A. anguilla**
	S. lucioperca	
	P. carbo	L. lutra

As an example of a fish predator for both zooplankton and zoobenthos we collected roach Rutilus rutilus, a dominant species of the Cyprinidae. Smelt, Osmerus eperianus, was collected as a species exclusively feeding on zooplankton. The polyphagous eel Anguilla anguilla preys on Chironomidae, Bivalvia, and small planktivorous Pisces (De Nie 1982). Finally, pike-perch, Stizostedion lucioperca almost exclusively feeds on fish (Bergers 1991). Adults are considered to be top-predators, too large to be eaten by piscivorous birds and mammals.

Several mollusci-, insecti- and piscivorous birds and mammals feed on the taxa sampled. Sand martin, Riparia riparia and several bats, Chiroptera, feed on pupated Chironomidae. D. polymorpha is an important food source for pochards, Aythya. Cormorant, Phalacrocorax carbo, largely feeds on Cyprinidae and Percidae species. The absence of otter, Lutra lutra, which feeds for 25% on eel, A. anguilla (Broekhuizen and de Ruiter-Dijkman 1988) is especially associated with chlorobiphenyl pollution.

3.2. Methods

3.2.1. Water and sediment concentrations

The Institute of Inland Water Management and Waste Water Treatment nowadays monitors contamination of water by lipophilic compounds as the concentration adsorbed to suspended particles (Venema 1991, Hendriks 1993). In this paper, we will compare residues in biota with suspended solids concentrations monitored at the nearest upstream location. Markermeer residues are related to suspended solid concentrations measured at the IJsselmeer monitoring spot, residues of Haringvliet to the suspended solid concentrations at Haringvliet and the other samples to data from the monitoring station at Lobith. This is illustrated by Figure 10. The average of sediment concentrations measured during 1985-1989 in each region is also taken into account. Concentrations are normalized to standard sediment and suspension consisting of 10% and 20% organic matter respectively. Concentrations at suspended solids and sediments will be expressed per $\mu g \cdot kg^{-1}$ dry organic matter in case of organic microcontaminants per $\mu g \cdot kg^{-1}$ dry matter in case of metals. More extensive discussions on variation of contamination in time, space and among compounds can be found in another paper (Hendriks 1993).

3.2.2. Organism concentrations

In accordance with the goal of the Rhine Action Program, this study aims to provide a general impression of current accumulation levels of some 20 compounds in 7 groups of organisms at 7 locations.

Throughout the summer of 1990, each taxon was sampled once at the location and in the period expected to yield the largest amounts. Even then it turned out to be difficult to sample enough material at some locations. In particular, invertebrates were hard to collect in quantities large enough for complete chemical analyses above detection limits. We aimed to capture different taxa close to each other, but if the amount collected was insufficient, a wider range was sampled. A complete analysis in duplicate required approximately 140 g wet weight. Fish were usually caught into sufficient amounts, ranging from 10 to 20 individuals. Adults with a length of 30 - 40 cm for A. anguilla and 40 - 50 cm for S. luciopercus were selected to be analyzed. R. rutilus was divided in three length classes. In this paper we will use the average of juveniles and adults. Since concentrations in both sediment and biota may show substantial variability within short periods and distances and within the same taxon, small differences will be neglected. Fluctuations in concentrations during summer are largely unknown and might hamper analysis but Kraak et al. (1991) reported seasonal differences of heavy metal concentrations in D. polymorpha in uncontaminated lakes to be a factor 3 or less.

The analysis of chlorobiphenyls and other organochlorine compounds consisted of drying 5 to 50 g (depending on expected concentration and lipid content) with Na_2SO_4 followed by soxhlet extraction with dichloromethane/n-pentane for 15 h. The extract was cleaned up over an alumina column and fractionated on a silica column. Determination was carried out by gas chromatography using an ECD.

Mercury and arsenic analysis were performed by cold vapor atomic absorption spectrometry (CVAAS). Lead and cadmium were determined by differential pulse anodic stripping voltammetry (DPASV), copper and zinc by graphite furnace atomic absorption spectrometry (GFAAS), respectively flame atomic absorption spectrometry (FAAS). Polyaromatic hydrocarbon analysis was performed using HPLC with fluorescence detection.

With the exception of polycyclic aromatic hydrocarbons measurements, chemical analysis was carried out in duplicates. In more than 90% of the cases, deviation between duplicates turned out to be less than 20%. Larger differences especially occurred in species of lower trophic levels and in β-hexachlorocyclohexane. Pieters (1991) describes further details.

Lipophilic microcontaminants tend to accumulate in the fat of organisms, so that the concentrations per wet weight are considered linearly proportional to the fat fractions (e.g. Mackay 1982). To stress

differences in locations and species rather than those in lipid fractions, we will express concentrations of organic micropollutants in $\mu g \cdot kg^{-1}$ fat weight. Heavy metals may accumulate throughout several components of organisms and will be given in $\mu g \cdot kg^{-1}$ dry weight. Concentrations below detection limits will be reported as half the detection limit.

3.2.3. Reference concentrations

Consumption standards offer a first impression of more or less safe concentrations. Some of the standards applied or proposed in The Netherlands are summarized in Table 2.

Table 2. (Proposed) consumption standards for animals (Stortelder et al. 1991), man (Van der Valk 1989) and birds and mammals (Romijn et al. 1991, Van de Plassche et al. 1991).

microcontaminant	(proposed) standards		
	animals	man	birds mammals
	$\mu g \cdot kg^{-1}$ wet weight		
Cd	$5.0*10^{1}$	$5.0*10^{1}$	$1.6*10^{2}$
Hg	$1.0*10^{3}$	$1.0*10^{3}$	$4.0*10^{2}$
HCB	$2.0*10^{2}$	$5.0*10^{1}$	$5.0*10^{2}$
PCBs	$1.0*10^{2}$	$2.0*10^{3}$	
PCB153	$1.0*10^{1}$	$1.0*10^{2}$	$1.0*10^{3}$
DDD+DDE	$1.5*10^{2}$	$5.0*10^{2}$	$1.3*10^{2}$
dieldrin	$3.0*10^{2}$	$5.0*10^{1}$	$1.0*10^{2}$
HCH	$2.0*10^{2}$	$1.5*10^{2}$	$1.6*10^{2}$

The first set consists of product standards, collected by Stortelder et al. (1991) and Van der Kooij et al. (1991), which were used for setting water quality standards. Stortelder et al. (1991) derived lower standards for both cadmium and for hexachlorobenzene. This was because exposure to water was considered more important for cadmium, while the hexachlorobenzene standard was corrected for combined exposure of all chlorobenzenes. As we focus on accumulation we took the original product standard collected for these compounds.

Van der Valk (1989) reviewed standards for fishery products from several countries. Omitting differences in water contents and within chemical groups, we inserted the lower values in Table 2. Concentrations in food, thought to be safe for most but not all bird and mammal species, have been estimated from various diet studies with a few laboratory species (Romijn et al. 1991, Van der Plassche et al. 1991). As this branch of ecotoxicology has only just begun to develop quantitative relationships, no "safe levels" for all or specific (semi-)aquatic birds and mammals can be derived. From Table 2. one may learn that application of various methods and data sets on different species yields quite different values. The subject of this article is to compare field concentrations with levels thought to be safe for most species, rather than to argue the validity of these procedures of extrapolation. Surprisingly, for each compound, the lowest of all (proposed) standards is close to $1*10^{2}$ $\mu g \cdot kg^{-1}$ wet weight in food. One may use this value as an impression of a low degree of damage rather than focus on relatively small variations for combinations of compounds and species that may partly be generated by differences in availability of knowledge. Using typical values of Table 3., the reference value of $1*10^{2}$ $\mu g \cdot kg^{-1}$ wet weight may be converted to $5*10^{2}$ $\mu g \cdot kg^{-1}$ dry weight and $2*10^{3}$ $\mu g \cdot kg^{-1}$ fat weight for an average animal consisting of 20% dry matter and 5% fat.

3.3. Results and discussion

3.3.1. General

Table 3. demonstrates that the dry to wet weight fraction is approximately 5% for Chironomidae, 10% for Crustacea and Bivalvia and 20% for all fish species except A. anguilla. The fat to dry weight fraction approximates 15% for most taxa. Half of the dry biomass of A. anguilla consists of fat reserves. Only 4% of the other top-predator, S. lucioperca, consists of fat. Thus, the fat to wet weight fraction ranges from 1% for S. lucioperca, 3% for R. rutilus and O. eperianus to 20% for A. anguilla.

Table 3. Average dry to wet ($p_{dry:wet}$) and fat to dry ($p_{fat:wet}$) weight fractions of aquatic animals with minimum and maximum between brackets.

taxon	fraction	
	$p_{dry:wet}$	$p_{fat:dry}$
	%	
Crustacea	9 (3-15)	15 (10-27)
Bivalvia	12 (8-13)	14 (6-28)
Chironomidae	5 (2- 7)	13 (9-42)
O. eperlanus	18 (16-19)	14 (9-21)
R. rutilus	24 (19-29)	12 (6-23)
A. anguilla	38 (31-45)	53 (48-68)
S. lucioperca	23 (19-23)	4 (3- 9)

Residues of most organic micropollutants increase slightly in a downstream direction from Lobith to Ketelmeer in the north and Haringvliet-East in the west. The most downstream locations, Markermeer and Haringvliet-West are clearly less polluted. This series is in agreement with concentrations in sediment and suspended solids measured between 1985 and 1990 (Hendriks 1993).

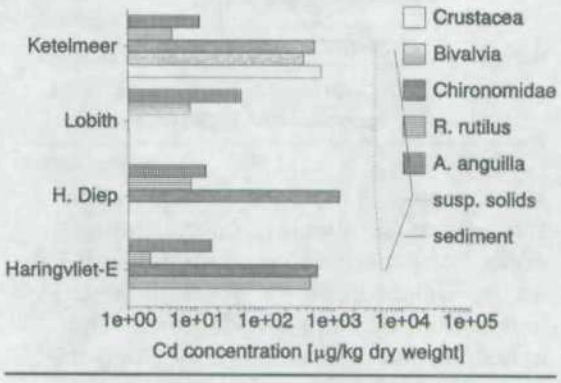
On the average, concentrations of organic compounds in fish fat are on the same level as those in/at organic sediment. The same applies to inorganic compounds in total fish and total sediment if expressed per dry weight. Contamination in fish is generally twice as high as in invertebrates. We will now elaborate this general picture.

3.3.2. Cadmium

The cadmium residues in invertebrates and fish are plotted in Figure 11. The levels in invertebrates tend to decrease in a downstream direction from Lobith to Ketelmeer in the north and to Hollands Diep and Haringvliet in the west. Salomons (1989) attributed this to the increase of pH, which reduces the dissolved -and therefore available- fraction of cadmium. In the German part of the river cadmium concentrations in D. polymorpha increased steadily, with a maximum in the Dutch river branches (Van der Valk et al. 1989).

The concentrations in Bivalvia are higher than the 1 $\mu g \cdot kg^{-1}$ dry weight measured by

Figure 11. Cadmium concentrations in biota dry weight and sedimented (suspended) dry weight from the Rhine delta in 1990. Some Chironomidae residues were collected in another investigation (Anonymous 1991).

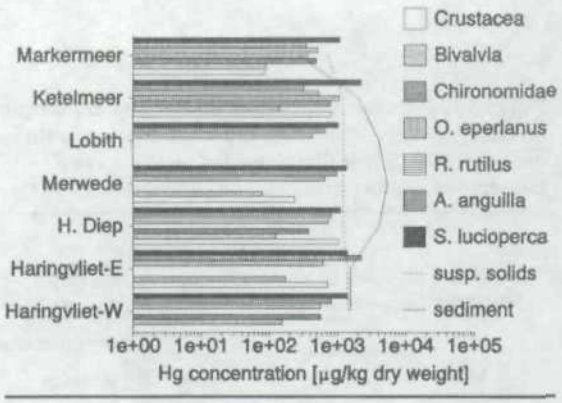


Kraak et al. (1991) in D. polymorpha of uncontaminated lakes, among which lake Markermeer. Cadmium accumulation in the same species at Lobith decreased from $7.4*10^4$ to $1.5*10^3$ $\mu g \cdot kg^{-1}$ dry weight between 1976-1988 (Kraak et al. 1991), which coincided with a similar sharp trend in total water concentrations (Hendriks 1993). Figure 11. shows that all residues measured in this study at other locations are below this level. Cadmium accumulation in invertebrates is 3 to 5 times more severe in Hollands Diep than in Ketelmeer (Anonymous 1991). This is reflected in sediment and less clearly in fish concentrations. Figure 11. demonstrates that cadmium concentrations in sediment (suspension), invertebrates and fish are about $5*10^3$, $5*10^2$ and $1*10^1$ $\mu g \cdot kg^{-1}$ dry weight. All fish samples do meet the reference value of $5*10^2$ $\mu g \cdot kg^{-1}$ dry weight but most invertebrate residues exceed this level.

3.3.3. Mercury

Variation of mercury concentrations in the Rhine delta in both organisms and sediment fits in the general pattern already described. Van der Valk et al. (1989) concluded that D. polymorpha suffers the

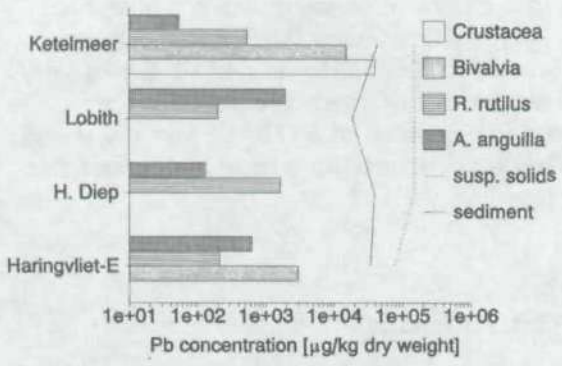
Figure 12. Total mercury concentrations in biota dry weight and sedimented (suspended) dry weight from the Rhine delta in 1990.



same degree of mercury contamination throughout Germany. In contaminated areas, total mercury concentrations in fish on dry weight basis are generally 3 times higher than those in invertebrates. The concentration of total mercury along the food chain is in proportion of 0.5 : 1 : 4 for invertebrates, young and old fish respectively. The general reference value of $1*10^2$ $\mu g \cdot kg^{-1}$ wet weight or $5*10^2$ $\mu g \cdot kg^{-1}$ dry weight is often exceeded in fish samples but these levels meet the less severe human consumption standard of $1*10^3$ $\mu g \cdot kg^{-1}$ wet weight.

3.3.4. Lead and copper

Figure 13. Lead concentrations in biota dry weight and sedimented (suspended) dry weight from the Rhine delta in 1990.

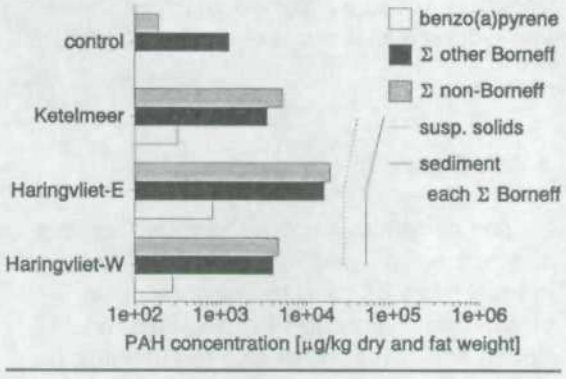


The level of lead contamination in invertebrates and fish of Figure 13. is somewhat above consumption standards and ranges from $5*10^2$ to $2*10^3$ $\mu g \cdot kg^{-1}$ wet weight. Copper concentrations in D. polymorpha at Lobith were stable on a level of 20 to 25 $\mu g \cdot kg^{-1}$ dry weight during the period 1976-1988 (Kraak et al. 1991). In the same period total water concentrations declined by a factor 3 (Hendriks 1993).

3.3.5. Polycyclic aromatic hydrocarbons

Concentrations of most polycyclic aromatic hydrocarbons do not increase along food chains (Biddinger and Gloss 1984). Thus, polycyclic aromatic hydrocarbons were only measured in invertebrate samples large enough to assess concentrations of magnifying compounds as well. At some locations, Bivalvia turned out to be present in amounts large enough for analysis of these compounds. The data given in Figure 14. do not coincide with sediment contamination, but this may be due to the scarcity of data. Nevertheless, Figure 14. indicates that accumulation in the Rhine delta Bivalvia is higher than the level in a small control lake (Anonymous 1991).

Figure 14. Polycyclic aromatic hydrocarbon concentrations in Bivalvia fat weight and organic sedimented (suspended) dry weight from the Rhine delta in 1990.



Some polycyclic aromatic hydrocarbons and their metabolites have a mutagenic and/or carcinogenic potential but knowledge on safe concentrations is scarce. If one converts the quality standard for benzo(a)pyrene of $1*10^3$ $\mu g \cdot kg^{-1}$ wet weight (Stortelder et al. 1991, Canadian Council 1987) to Bivalvia that contain 2% fat one obtains a reference concentration of $5*10^4$

$\mu g \cdot kg^{-1}$ fat weight. In Haringvliet-Oost benzo(a)pyrene residues in Bivalvia are approximately 50 times lower, so that poisoning via the food chain seems unlikely for this compound alone. The importance of accumulation of other Borneff polycyclic aromatic hydrocarbons, viz. benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, fluoranthene and indeno(1,2,3-c,d)pyrene cannot be assessed.

The degree to which concentrations in Figure 14. are harmful to the organism itself is also difficult to estimate. Internal concentrations are seldom recorded and cannot be derived from external concentrations since elimination seems specific for combinations of compounds and species.

3.3.6. Monocyclic chloroaromatic hydrocarbons

Some monocyclic chloroaromatic hydrocarbons are known to accumulate and penta- and hexachlorobenzene are best known in this respect. Accumulation of chlorobenzenes increases with the degree of chlorination. Since pentachlorobenzene concentrations were generally 2 to 10 times lower than those of hexachlorobenzene we will confine ourselves to the latter compound only.

Figure 15. Hexachlorobenzene concentrations in biota fat weight and organic sedimented (suspended) dry weight from the Rhine delta in 1990.

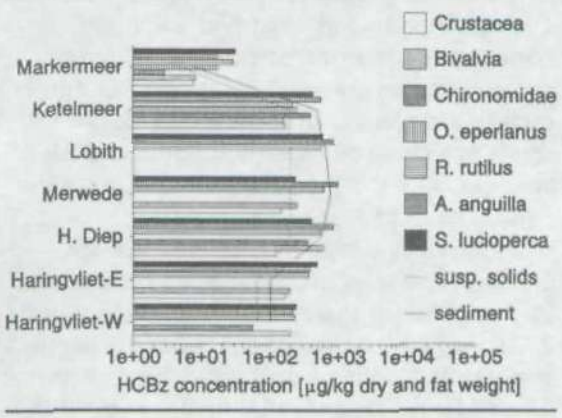


Figure 15. shows that hexachlorobenzene contamination in organic suspended solids, organic sediment, invertebrate fat and fish fat is in the proportion of 0.3 : 1.2 : 0.6 : 1 respectively. The reference value of $2*10^3$ $\mu g \cdot kg^{-1}$ fat weight is not exceeded in any sample. Only A. anguilla from highly contaminated areas contains more hexachlorobenzene than allowed by consumption standards, if applied to wet samples.

3.3.7. Chlorobiphenyls

On average, chlorobiphenyl concentrations in organic suspension, organic sediment, invertebrate and fish fat are in the proportion of 0.1 : 0.9 : 0.5 : 1. Residues of higher chlorinated chlorobiphenyls in fish and invertebrates differ by approximately a factor 3. This is in line with a less than 3-fold difference between PCB153 concentrations in A. anguilla and phytoplankton measured in a Dutch freshwater lake by Van der Oost et al. (1988). More specifically, concentrations in Chironomidae from the highly contaminated lakes Hollands Diep and Ketelmeer were higher than those in Crustacea and Bivalvia. The reverse occurred in less contaminated Haringvliet-West and Markermeer. In our study, PCB028, PCB052, PCB101, PCB118, PCB(138+163), PCB153, PCB180 generally contributed 2%, 9%, 13%, 10%, 20%, 36%, and 11% to the internal total PCB concentration of Figure 16., but lower chlorobiphenyls were present at much lower concentrations in A. anquilla (Pieters 1991).

After a sharp decrease in the early eighties the decline of chlorobiphenyls concentrations in abiotic and biotic compartments now seem to level off. This tendency is most apparent for higher chlorinated congeners in organisms as illustrated by Figure 17.

Recently, non-ortho (PCB77, PCB126 and PCB169) and mono-ortho (PCB105, PCB118, PCB156) chlorobiphenyls have turned out to be more important than the regularly measured standard set, because these have properties similar to those of chlorodibenzodioxins. Figure 18. contains preliminary data of non-ortho chlorobiphenyls by De Boer and Hagel (1992) and residues of mono-ortho

Figure 16. Chlorobiphenyl 28+52+101+118+138+153+ 180 concentrations in biota fat weight and organic sedimented (suspended) dry weight from the Rhine delta in 1990.

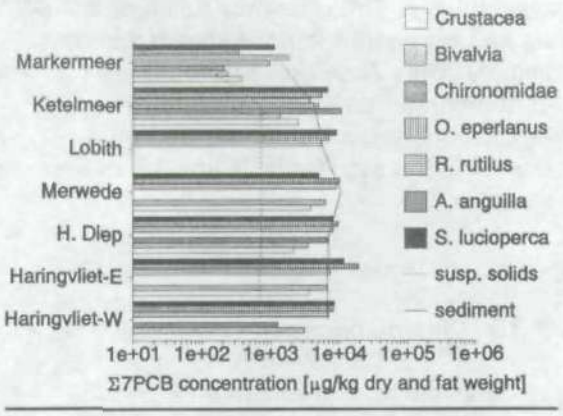
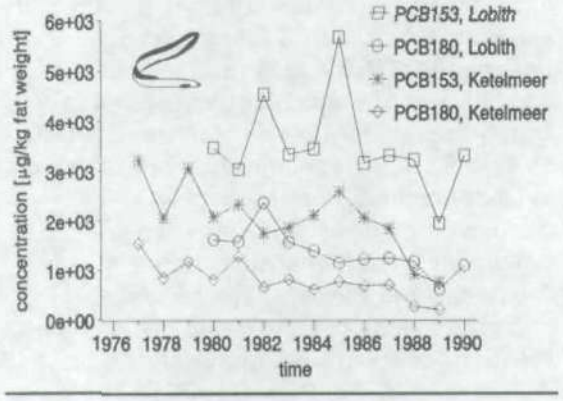


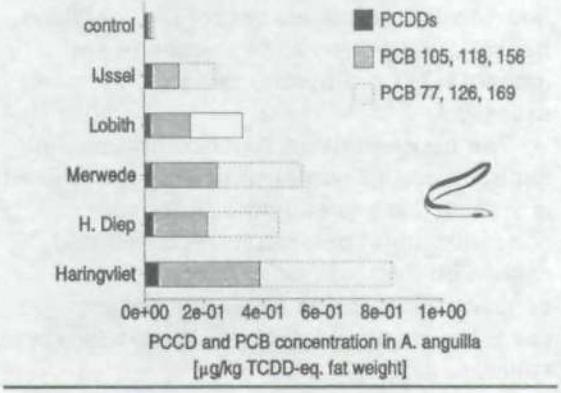
Figure 17. Chlorobiphenyl 153 and 180 concentrations in eel, A.anguilla fat weight at Lobith and lake Ketelmeer during the period 1976-1990 (De Boer and Hagel 1992)



chlorobiphenyls, chlorodibenzodioxins and -furanes by Hagel (1990) in eel, A. anguilla.

In Figure 18., Lauwersmeer is added as a control location. Non-ortho chlorobiphenyls were only measured at Lobith, values for other locations were calculated in proportion to the mono-ortho chlorobiphenyls (dashed lines). All concentrations are expressed in 2,3,7,8-tetrachlorodibenzodioxin (TCDD) equivalents (Ministry of Public Housing and Environmental Protection 1990). Total TCDD-equivalent values in A. anguilla from the Rhine delta varied by a factor of 5, but were at a level 20 to 100 times higher than those for the less contaminated lake Lauwersmeer. Dioxin-like toxicity of the "toxic" chlorobiphenyls, expressed as TCDD-equivalents, accounted for more than 80% of the total TCDD-equivalents in yellow eel from the Rhine delta.

Figure 18. Mono- and non-ortho chlorobiphenyl and dioxin concentrations in eel, A. anguilla fat weight *from the Rhine delta in 1990. Dotted lines are* extrapolations for the fraction measured at Lobith.



On-going debates have not yielded widely accepted standards for these compounds. Some indicative values (Turkstra and Pols 1989, Canadian Council 1987) are in the range of tens of $ng \cdot kg^{-1}$ wet weight TCDD equivalents. Concentrations of mono-ortho- and non-ortho chlorobiphenyls and dioxins of Figure 18. exceed this level. The same holds for concentrations of chlorodibenzodioxins and -furanes in some fish samples from the Rhine delta (Turkstra and Pols 1989).

3.3.8. Chlorobiocides

Hendriks (1993) reported that measured average suspended solid and sediment concentrations of most traditional chlorobiocides are now below or just above quality standards. Unfortunately, these levels are close to detection limits, which also explains the missing sediment data in Figure 19. and Figure 21.

Figure 19. demonstrates that dieldrin concentrations in organisms are on average 20 times lower than the reference value of $2*10^3$ $\mu g \cdot kg^{-1}$ fat weight. These low levels endorse the conclusion by Biddinger and Gloss (1984) that residue levels of dieldrin will drop after reduction of immission. As dieldrin is a metabolite of aldrin degradation, aldrin contamination will probably be even less severe.

Figure 19. Dieldrin concentrations in biota fat and organic suspended weight from the Rhine delta in 1990.

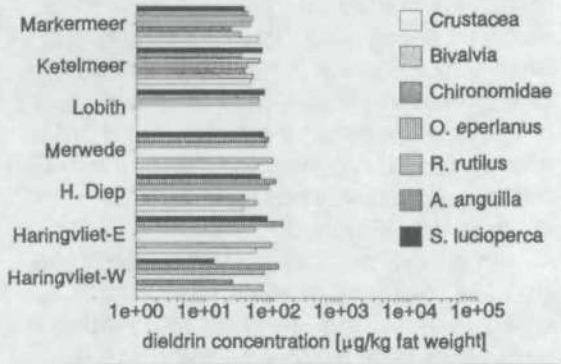
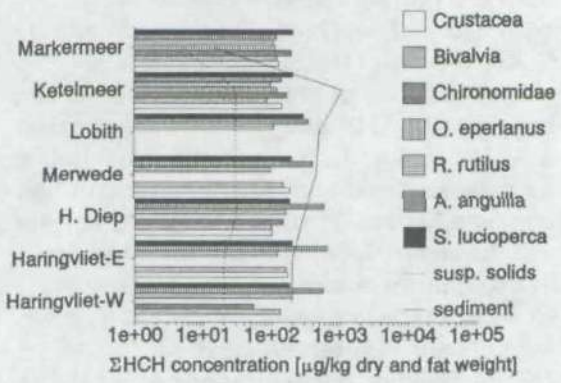


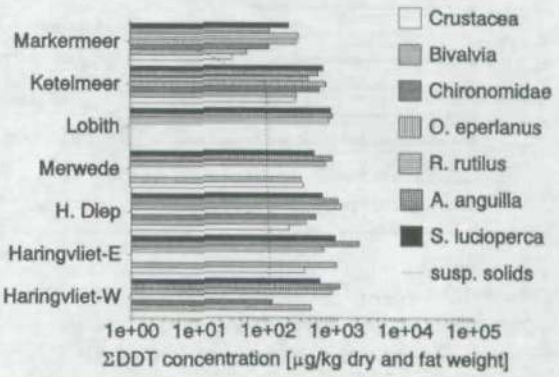
Figure 20. Hexachlorocyclohexane concentrations in biota fat and organic sedimented (suspended) dry weight from the Rhine delta in 1990.



Apart from the A. anguilla samples, most of the residues met the reference value for hexachlorocyclohexane. On the average, concentrations in organic suspended solids, organic sediment, invertebrates and fish were in the proportions of 0.1 : 2 : 0.6 : 1. The contribution of α-, β-, γ-isomers to the total hexachlorocyclohexane concentration was 20%, 55%, 25% respectively in sediment (Hendriks 1993), and 10%, 20% and 70% respectively in invertebrates and fish.

The total (DDX) concentration of dichlorodiphenyltrichloroethane (DDT) and its derivatives (DDD and DDE), in organisms are close to reference levels. The relative accumulation concentrations in organic suspension, invertebrate and fish fat were 0.1 : 0.4 : 1. Reference values for DDX compounds in Table 2. refer to DDE and DDD, because DDE, DDD and DDT contributed respectively about two-third, one-third and less than 2% to the total DDX

Figure 21. Dichlorodiphenyltrichloroethane-(derivatives) concentrations in biota fat and organic suspended dry weight from the Rhine delta in 1990.



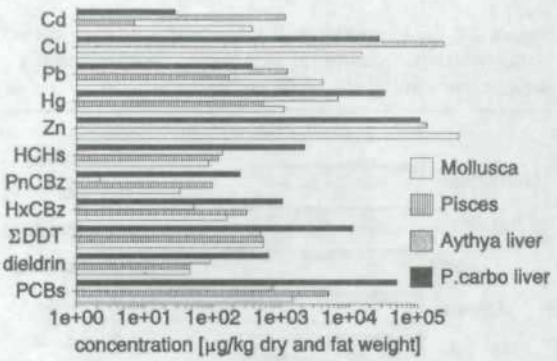
in our samples.

3.3.9. Birds and mammals

Interpretation of concentrations in birds and mammals at the end of the aquatic and terrestrial food chain in the Rhine delta is hampered because of migration. In contrast to lower taxa, residues in birds and mammals are often measured in specific organs. Large variations in the liver:muscle ratios exist. For mercury, this ratio ranged between 0.9 and 2 in fish (RIVO 1991) and 0.2 to 3 in floodplain cattle (Van der Ven et al. 1977). Ratios for chlorobiphenyls in fish vary between 1 and 2.5. This may hold for several chlorinated compounds, but in birds a large accumulation in (affected) specimen have been found (Koeman et al. 1973, Walker 1990). Van Eerden (personal communication) investigated residues in birds that thrive near lake Ketelmeer during 1990 and 1991. The average contamination level of 18 livers of pochards, Aythya, and - for organic compounds- 20 livers of cormorants, P. carbo are compared to average food residues in Figure 22. The internal concentrations of cadmium, copper, lead and zinc have been analyzed in two cormorants livers only.

These birds are known to migrate over small and large distances. Ketelmeer is one of the most contaminated large lakes in The

Figure 22. Organic microcontaminant [$\mu g \cdot kg^{-1}$ fat weight] and heavy metal [$\mu g \cdot kg^{-1}$ dry weight] concentrations in mollusc or fish muscle and bird liver weight at lake Ketelmeer in 1990/1991.



Netherlands, so that differences between concentrations in bird livers and food in Figure 22. reflect a minimum if uptake abroad is excluded.

Despite these complications, the pattern of Figure 22. agrees with other papers on accumulation. Heavy metals concentrations in Aythya tend to be somewhat higher or lower than in Mollusca. The conclusion by Biddinger and Gloss (1984) that cadmium accumulation in invertebrates is important, is confirmed by the increased levels in Mollusca and their predators Aythya. However, the cadmium, lead and mercury residues in these birds do not exceed the background level of about $5*10^3$ $\mu g \cdot kg^{-1}$ dry weight mentioned by Scheuhammer (1987). The accumulation of copper is clearly increased compared to the levels in Mollusca. Mercury accumulation is higher in the livers of piscivorous cormorants, compared to those of the molluscivorous pochards.

Organic contaminants accumulate in the highest order consumer P. carbo, particularly those that are more persistent. Geometric averaged concentration ratios of mercury, higher chlorobiphenyls, DDE, dieldrin, hexachlorobenzene in cormorant liver and fish were in the proportion of 60, 10-20, 30, 15 and 3. In 1970, Koeman et al. (1973) collected several cormorants, P. carbo. In livers of 3 animals shot at Naardermeer they measured accumulation of these compounds that was 4 (DDE) to 14 (PCBS) times higher than those of Ketelmeer in 1990. Residues in a set of six birds found dead throughout The Netherlands in 1970, were at most 6 times higher, with the exception of hexachlorobenzene. Koeman et al. (1973) concluded that the chlorobiphenyls residues measured in their study are likely to cause death of cormorants. Ignoring details on life history of individual cormorants in each study, one may conclude that a decline in residues -if present- is less than one order of magnitude. A similar low decline is found for higher chlorobiphenyls in fish, as illustrated by Figure 17.

In his review, Walker (1990) concludes that liver residues of more than $2*10^5$ $\mu g \cdot kg^{-1}$ wet weight are likely to be lethal to birds. This level is close to the residues given in Figure 22. after conversion to fat weight.

During the period 1982 to 1987, 4 specimens of the (almost) extinct population of otters, Lutra lutra, in the northern part of The Netherlands were collected and analyzed. Broekhuizen and de Ruiter-Dijkman (1988) report liver and kidney residues of chlorobiphenyls that vary between $3.4*10^3$ and -for a diseased male- $2.4*10^5$ $\mu g \cdot kg^{-1}$ fat weight. Their habitat is partially supplied with Rhine water and fish contained about $1*10^3$ to $1*10^4$ $\mu g \cdot kg^{-1}$ fat weight chlorobiphenyls. The minimum level in fish and the maximum level in otter are close to the averages of Figure 22. The lower levels in otter are close to chlorobiphenyls concentrations of $5*10^3$ to $5*10^4$ $\mu g \cdot kg^{-1}$ fat weight found in fat tissue of otter, Lutra canadensis, trapped throughout New York State (Foley et al. 1988). Liver concentrations of chlorobiphenyls above $1*10^3$ $\mu g \cdot kg^{-1}$ wet weight (Wren 1991) tend to affect Mustelidae. Occasionally, lower values are reported, such as about $5*10^2$ $\mu g \cdot kg^{-1}$ wet weight after consumption of food containing $2.5*10^2$ $\mu g \cdot kg^{-1}$ wet weight (Den Boer 1983).

Terrestrial food chains in the Rhine delta floodplains have hardly been investigated. Ma and Broekhuizen (1989) found cadmium residues in badger, Meles meles, along floodplains of the river Meuse that exceeded critical levels. On the other hand, Van de Ven et al. (1977) concluded that accumulation of heavy metals in Rhine delta cattle is probably of minor importance. As in aquatic communities, concentrations of heavy metals do not seem to increase very much along terrestrial foodchains (e.g.

Laskowski 1991, Hunter and Johnson 1982).

Fuchs and Thissen (1981) studied contamination in eggs of the carnivorous little owl, Athene noctua, and the omnivorous magpie, Pica pica, in Rhine delta floodplains. Concentrations of dieldrin, hexachlorobenzene, DDE and chlorobiphenyls in both species varied around 20, 50, 200 and over 1000 $\mu g \cdot kg^{-1}$ wet weight respectively. Assuming 5% fat in eggs, these values are roughly in the same order of magnitude as those in liver fat of Ketelmeer cormorants. Chlorobiphenyl residues decreased if the distance of nest sites to the river was increased.

3.4. Conclusions and recommendations

This study provided accumulation trends of some 20 microcontaminants in space, time and foodchains throughout the Rhine delta. To overcome the lack of costly multiple sampling -inherent to applied research- observed trends were compared to other information. Together, these yield the following picture.

Over the past decades residues of some traditional compounds have decreased substantially, but during the last decade the decline is suspected to level off. This coincides with similar trends of contamination in abiotic compartments and may reflect the decreasing yields of additional emission reduction measures. Residues of other compounds did not decline at all. This trend is most striking for PCB153 and PCB180 in the eel, A. anguilla. The importance of chlorobiphenyls is also stressed by preliminary data on "dioxin-like" PCBs. Moreover, concentrations of DDT and its derivatives are usually below detection limits in sediments, but these compounds still accumulate substantially in organisms. The general reference value of $1*10^2$ $\mu g \cdot kg^{-1}$ wet weight is exceeded in samples for cadmium, mercury and chlorobiphenyls. Residues of other compounds are below this reference value. The difference between actual and reference concentration increases from dichlorodiphenyltrichloroethane (DDT) and its derivatives (DDD and DDE), to hexachlorobenzene, hexachlorocyclohexanes and dieldrin. This suggests that an impact of micropollutants on species of the aquatic community cannot be excluded.

Concentrations of most traditional micropollutants in aquatic taxa tend to increase slightly in a downstream direction but the most downstream location of both branches is clearly less polluted.

On the average, concentrations of organic compounds in organic suspended solids, organic sediment, invertebrate and fish fat are in the proportion of 0.1 : 1 : 0.5 : 1. Residues that are somewhat higher than reflected by these ratios have been observed for chlorobiphenyls in fish and mercury in pike-perch, S. lucioperca. The taxa sampled represent three trophic levels so that the predator-prey accumulation ratio is 2 or less. This ratio was also noticed by Thomann (1989) in his review on ratios of fat-corrected residues of organic compounds in predator and prey fish. Though complicated by impact of migration and variability among organs, residues in birds are probably at least one order of magnitude higher than in fish.

Combining the foregoing we arrive at the following recommendations. As stagnation of downward trends cannot be excluded, monitoring should continue. Our results show that accumulation in aquatic species generally differs less than a factor 3. For a rather cheap and general impression of accumulation in aquatic species, we suggest analysis of heavy metals and polycyclic hydrocarbons in lower taxa, preferably Bivalvia, and mercury and persistent chloroaromatic hydrocarbons in one of the fish species. Substantial accumulation by other compounds not covered in this paper cannot be excluded and deserves attention in experimental studies. The same holds for species, especially those that occupy niches not represented in this paper. Scarce data on accumulation in birds and mammals demonstrate that residues in more terrestrial species deserve more attention in future. Some combinations of compounds and species, such as "dioxin-like" chlorobiphenyls and fish, are ready for more regular monitoring programs.

If current levels of pollution in the Rhine delta are compared to critical values, one has to conclude that a return of indigenous

species cannot be guaranteed. If pollution decline is indeed levelling off, more subtle knowledge of "safe" levels becomes crucial. Therefore attention should shift to *toxicological studies on relevant taxa.* Especially reviews like those by Scheuhammer (1987), Wren (1991), Walker (1990) and others are important for assessing taxon specific requirements.

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4. MONITORING RESPONSE OF XAD-CONCENTRATED WATER IN THE RHINE DELTA: A MAJOR PART OF THE TOXIC COMPOUNDS REMAINS UNIDENTIFIED

A. JAN HENDRIKS[1], JOHANNA L. MAAS-DIEPEVEEN[1], ARIE NOORDSIJ[2], MARTEN A. VAN DER GAAG[3]

[1]Institute for Inland Water Management and Waste Water Treatment, RIZA, P.O. Box 17, 8200 AA, Lelystad,
[2]Netherlands Waterworks Testing and Research Institute KIWA NV, P.O. Box 1072, 3430 BB Nieuwegein,
[3]present address: Ministry of Housing, Physical Planning and Environment, P.O. Box 30945, 2500 GX Den Haag, The Netherlands

Abstract

In this study a part of the organic compounds present in Rhine water was isolated by XAD-resins and fractionated. Isolates as well as fractions were tested for mutagenicity and toxicity.

The highest mutagenic effects in the Ames test were observed with Salmonella typhimurium strain TA98 in the pH 7 isolate. Comparison of past data showed that mutagenicity remained the same in the period 1980-1990. The water samples had to be concentrated at least 25 times by XAD to induce short-term mortality in waterfleas (Daphnia magna), which indicates a substantial improvement in comparison with pollution during the seventies. Chronic toxicity was observed in Daphnia magna after lower levels of XAD-concentration. Extrapolation of these results to field cladocerans is discussed.

Most mutagenicity was recovered in the moderately hydrophilic diethylether, ethylacetate, ethanol fractions, but toxicity was almost exclusively located in the lipophilic cyclohexane fraction. However, assuming concentration addition to be dominant in mixtures, the major part (more than 89%) of the toxicity in the cyclohexane fraction could not be attributed to the GC-MS identified compounds, for which EC50 values were obtained from databases. Several probable causes for this discrepancy are discussed. However, the major contribution lacking is expected to be from identified compounds for which no information was found in the databases or from compounds that could not be identified by GC-MS.

It is concluded that the emission reduction along the Rhine should continue, with a more important role for toxicological assays.

Our study did not cover metals, very hydrophilic or very lipophilic compounds.

Key words: organic micropollutants, toxicity, mutagenicity, XAD, Daphnia magna, Salmonella typhimurium, Rhine

4.1. Introduction

In the sixties and seventies, the Rhine was heavily polluted. Among other effects, its water caused acute death of water fleas and trout embryos as well as malformations and other effects in fish (Poels et al. 1980, Slooff 1982a, Slooff et al. 1985, Alink et al. 1980, Van der Gaag et al. 1983, Van der Gaag 1987). The pollution problem is of major concern in The Netherlands, because the country is at the downstream end of the river basin. Rhine water is a major source for drinking water and water for industry and agriculture. Surface water in low parts of The Netherlands may contain more than 50% of Rhine water. Yet, the basin is an area with both intensive industrial and agricultural production and consumption inducing large point and diffusive immissions into the Rhine.

As a result of the concern for this large-scale pollution the "Rhine Action Program" was started in 1987 (International Rhine Commission, 1987). Most of the work so far has focused on a set of relatively well-known priority pollutants. Yet, it is extremely important to obtain an impression of the toxicity of the thousands of largely unknown compounds that are also present in the water. In this paper, we aim to characterize or identify the groups of

compounds that are responsible for toxic effects, by linking chemical analysis and toxicity testing. So far, experimental constraints have inhibited widespread application of extraction techniques for simultaneous chemical analysis and toxicity testing (e.g. Noordsij et al. 1983), except for a comparable study by Galassi et al. (1992)

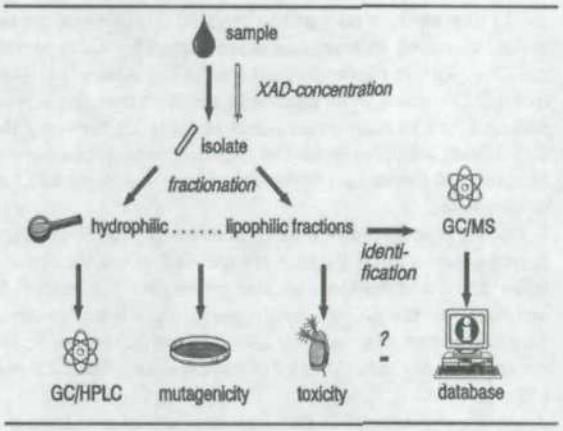
4.2. Methods

In 1989, we collected samples of Rhine water at several locations in The Netherlands delta. Samples were taken near the German border at Lobith (17.03.89 and 15.09.89), downstream of Rotterdam harbour at Maassluis (26.05.89 and 22.09.89), along the main stream at Werkendam (29.09.89), in the semi-stagnant branch at Haringvliet (19.05.89) and in the outlet of the IJssel at Kampen (21.04.89, Figure 23.). A water sample of Markermeer was taken as a reference (24.01.91). At each location 600 l. of water was taken in an 800 l. stainless steel tank and processed in the laboratory on the same day.

Figure 23. The Rhine delta and the sampling sites at the border (Lobith), halfway (Werkendam) and at the end of the branches (Kampen, Maassluis, Haringvliet). The reference site (Markermeer) is not influenced by the Rhine.

The organic matter was concentrated following the procedure by Noordsij (1983). After sedimentation of suspended matter, the 600 l. of water was led over two columns at a rate of one bed volume per minute (=600ml·min^{-1} ≡ 1000min = 16.6h), each filled with 600 ml of XAD-4 resin (Amberlite, Rohm & Haas, polystyrene 750 m^2·g^{-1}, pore diameter 50 Å). Adsorption was realized in the first column at the pH 7, and to the second at pH 2, after acidification with hydrochloric acid. The columns were subsequently eluted with 3.3 bed volume ethanol(=2l) and 3.3 bed volume(=2l) ethanol:cyclohexane (30%:70%) at 0.1 bed volume·min^{-1}(=60 ml·min^{-1}) (Baker, P.A., redistillated by KIWA). This was followed subsequently by azeotropic distillation with ethanol:cyclohexane:water (17%:76%:7%, at 62°C) and ethanol:cyclohexane (30.5%:69.5%, at 64°C). After evaporation, ethanol was concentrated to 24 ml (=25,000 times).

Figure 24. Overall scheme of the experimental methods used.



The mass-balance of the dissolved organic fraction was investigated after the different adsorption steps by DOC (Technicon instrument method 141-7-W) and u.v.-measurements (at 254 nm).

Fractionation of the XAD-isolates was carried out in five successive extraction steps with cyclohexane, diethylether, ethylacetate, ethanol and ethanol:water. The samples were finally concentrated in ethanol, at a concentration factor of 40 μl·l^{-1} of unconcentrated sample.

Both the isolates and the fractions were analyzed with GC (50 m, capillary fused filter, OV-1, 30°C→320°C, 5°C·min^{-1}) and HPLC. The reversed-phase HPLC-analysis was performed on a Microspher C18 column (Vydac 201 tpb). The column was eluted with a gradient of 100% eluent A (98% water, 0.5% triethylamine, 1.5% acetic acid) to 100% eluent B (acetonitrile) at a flow of 1 ml·min^{-1}, an injection of 5 μl

and with a variable wavelength detector Milton Roy spectroMonitor 3100 (wavelength 254 nm, sensitivity 0.01 and 0.1 AUFS at a wavelength of 10 nm).

The cyclohexane fraction, containing most of the compounds identified by gas chromatography, was additionally analyzed by mass spectrometry (TS250-VG, electron impact = 80 eV, scan speed = 2 s, m/e 35-450). The measured peak-intensities were converted to concentrations with the use of a reference compound with a high response factor which was added to the extract. Due to unknown differences in isolation efficiencies and response factors these concentrations may reflect a minimum.

The total isolate as well as each fraction was tested for mutagenicity in the Ames plate incorporation assay (Salmonella typhimurium). We used strains TA98 and TA100 without and with addition of rat hepatic metabolic fractions (S9-mix, Maron and Ames 1983, Van der Gaag 1988). Results from the mutagenicity assays are reported as the number of revertants per litre equivalent of non-concentrated water.

For short-term (2 d) toxicity testing, waterfleas (Daphnia magna) were exposed to isolates and fractions diluted by Dutch Standard Water (NNI 1980) according to a standard protocol (ISO 1989). The animal density in the replicate assays matched the required minimum of 10 $ml \cdot l^{-1}$ per 5 animals. Though ethanol was present in concentrations up to 10 $ml \cdot l^{-1}$, which is 100 times the maximum proposed by the protocol, this level was not toxic in controls.

To obtain an impression of the chronic toxicity, some preliminary long-term (21 d) assays with D. magna were carried out, according to the procedure described by Enserink et al. (1991). Because of the high ethanol concentrations, vessels were aerated for 5 min every day.

Toxicity will be expressed as the concentration factor (ECF50) that causes mortality or immobility in 50% of the rest organisms at the end of the exposure period.

For the compounds identified by GC-MS, we collected short-term (=1-4 d) response concentrations measured for waterfleas, mainly Daphnia magna and Daphnia pulex. We selected records with the review codes 1-3 from the AQUIRE-database (Pilli et al. 1989) and a few additional data the AQUATOX-database, also containing critically evaluated measurements (BKH 1990). If several values were obtained for a compound, we took the geometric mean of the set.

If no measurements were available, we estimated the minimum acute EC50 with a QSAR (=Quantitative Structure Activity Relationships model (Montana State University 1989).

4.3. Results and discussion

4.3.1. Chemical characteristics of Rhine water

4.3.1.1. Mass balance and physico-chemical characteristics of organic substances

Table 4. shows that about half the dissolved organic carbon in Rhine water was isolated with the combination of XAD-4 adsorption at ambient and acid pH. The estimates of the DOC agreed well with the u.v.-measurements.

Table 4. Recovery of dissolved organics with XAD from Rhine water is less than 50% The sample from Kampen shows a shift towards more hydrophilic compounds, compared to Lobith.

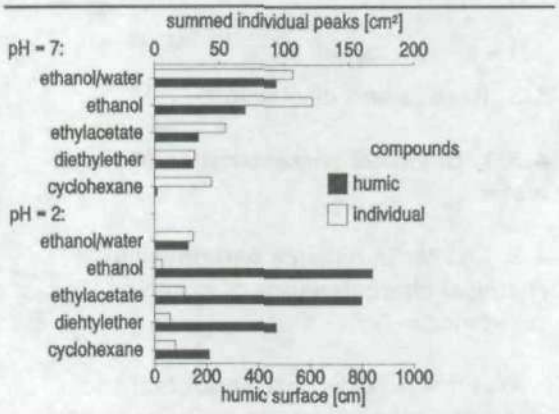
locations and parameters	XAD		left in outflow
	pH = 7	pH = 2	
Lobith, 17.03.1989			
DOC	31 %	20 %	49 %
UV	26 %	22 %	52 %
Kampen, 21.04.89			
DOC	15 %	35 %	50 %
UV	16 %	28 %	56 %
Haringvliet, 19.05.89			
DOC	25 %	nd	nd
UV	13 %	nd	nd

nd = not detected.

As illustrated by Figure 25. and summarized in Table 5., almost 85% of the isolated organic carbon consists of *humic compounds* that occur naturally and

probably will not harm the aquatic community. An analysis of the concentrated material with HPLC showed that 15% of the peak intensity of the pH 7 fraction, and 3% of the pH 2 fraction can be attributed to distinguishable peaks from individual compounds.

Figure 25. Summed intensities of humic and individual compounds in the autumn sample from Lobith measured by RV-HPLC. The XAD pH 7 fraction (relatively lipophilic) contained more individual peaks than the pH 2 fraction.



From these individual compounds, about 2% can be analyzed with GC-MS in the pH 7 fraction, and about 0.5% in the pH 2 fraction.

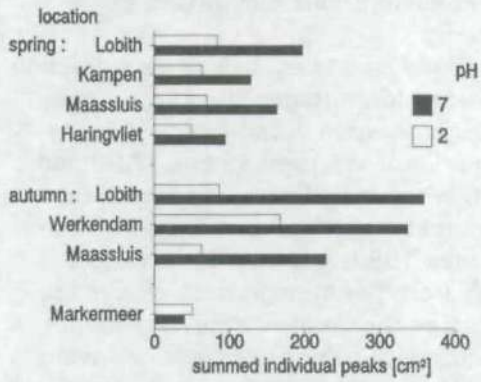
4.3.1.2. Chemical changes in Rhine water in the Rhine delta

Table 5. Summed intensities of humic and individual compounds, averaged for 8 locations.

compounds	summed intensities			
	pH = 7		pH = 2	
	cm^2	%	cm^2	%
humic compounds	1081	85	2775	97
individual compounds	194	15	80	3
of which GC-detectable	24	2	13	0.5

About 1000 individual compounds could be discerned in the GC-MS analysis, confirming findings from many previous chemical analyses of Rhine water (Puyker et al. 1989, Van Genderen and Noij 1991). Comparison between different samples is complicated because isolation recovery and specific responses of the mostly unidentified compounds are unknown, whereas differences between sites may be partly attributed to differences in time of sampling. Despite these uncertainties, a rough screening of the relative trend can be obtained from the summed peak intensities from HPLC (Figure 26.) and the summed concentrations in GC-MS analysis (Figure 27.).

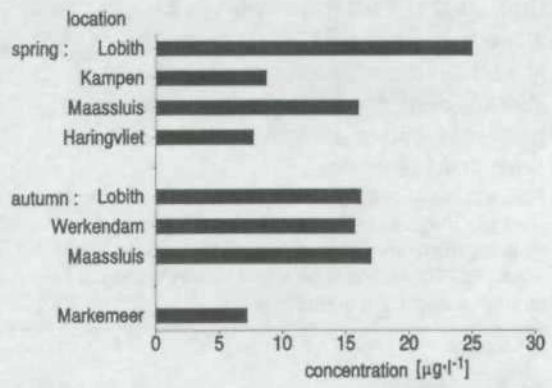
Figure 26. Intensities of individual compounds in with RV-HPLC (sum of 10 highest peaks). The highest levels were observed in the pH 7 fractions, in autumn and in main stream samples (Lobith, Werkendam, Maassluis).



The total amount of *individual substances*, measured as the summed intensity of individual peaks in HPLC-chromatograms tended to be highest in Lobith. The summed concentrations of individual compounds analyzed by GC-MS was also higher in the Rhine main stream (Lobith, Werkendam and Maassluis) compared with those in the IJssel branch (Kampen) or the semi-stagnant lake of Haringvliet.

Though the overall peak intensity appeared to be similar, some marked differences were observed for groups of compounds. In the September samples for instance, concentrations of nitrogen-compounds (pH = 7) and phenols (pH = 2) were respectively 1.5 and 2 times higher at Werkendam than at Lobith, whereas several non-identified peaks of halogenated aliphatic compounds found in Maassluis were not present in the other samples. This may mark a substantial input after Lobith, in case between sampling moments can be ignored. Differences for specific compounds and locations can be found in the Appendix.

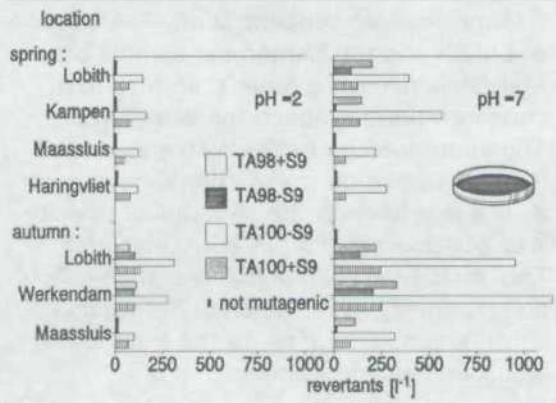
Figure 27. Summed concentrations of all compounds identified with GC-MS (peaks converted with reference compound). The data for Maassluis were corrected for dilution with sea water.



4.3.2. Genotoxicity in the Ames assay

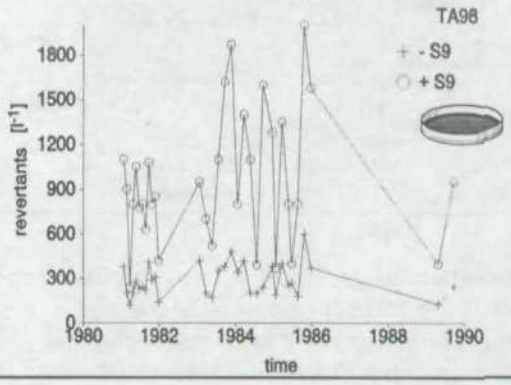
XAD-blanks and samples from the reference site of Markermeer did not cause mutagenic effects in the Ames assay. Yet, XAD samples from all locations along the Rhine induced genotoxicity in strain TA98, as illustrated in Figure 28.

Figure 28. Mutagenic response of bacteria in the Ames assay exposed to isolates from several locations. The highest levels are observed for TA98 + S9 strain in the pH 7 fraction of the autumn samples.



The addition of a *metabolic fraction of rat-liver (S9)* caused a marked increase of the mutagenic effect, in particular in the ambient pH XAD-isolate. The genotoxic effect of the pH 2 XAD-fraction was markedly lower than the neutral isolate, and the increase due to metabolic transformation was much smaller. Mutagenic effects in the TA100 strain occurred in the samples at Lobith and Werkendam, with about 100-200 revertants per litre induced in the pH 7 XAD-isolate without S9-mix, and 200-350 after addition of S9-mix. The pH 2 mutagenicity in TA100 was lower (100 revertants per litre), and S9-mix addition had no measurable effect.

Figure 29. Mutagenic response of bacteria (TA98) in the Ames test exposed to isolates from Lobith. Levels have not changed significantly between 1980 and 1990. Trends for pH 2 were similar (not shown).

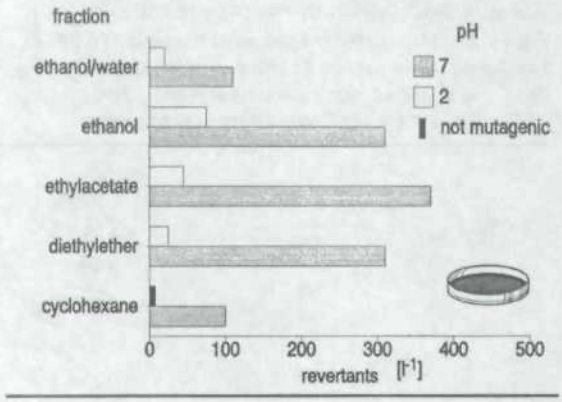


The findings of this study are in full agreement with the outcome from the regular monitoring program since 1981 in Figure 29., It shows that the TA98 frame shift mutagens that require hepatic metabolic activation are predominant in Rhine water. The occurrence of base-pair substituting mutagenic effects toward strain TA100 is low or sometimes even not detected. This pattern appears, although many discharged effluents are known to cause mutagenic effects in TA100. Similar patterns of genotoxicity were observed in other polluted rivers, such as the Meuse in north-western Europe, the Po in Italy (Galassi et al. 1992) and in the Yodo river in Japan (Nakamuro et al. 1991). Yet, the intensity of the mutagenic effect of Rhine water is still much higher than in other rivers.

Analysis of mutagenicity in the sub-fractions of the XAD-samples in Figure 30. showed that the major part of the mutagenicity is found in the diethylether, ethylacetate and ethanol fractions of the pH 7 XAD-isolate. A similar but less marked distribution was found in the pH 2 sample. The genotoxic effect in the cyclohexane-fraction of both pH 2 and pH 7 is small, which means that only a minor part of the genotoxins present can potentially be identified with GC-MS.

The ecological significance of genotoxicity to aquatic species is still an

Figure 30. Mutagenic response of bacteria (TA98+S9) in the Ames test exposed to different fractions in autumn at Lobith. Most of the response is recovered in the moderately (pH 7) to strongly (pH 2) hydrophilic extracts.



uncertain issue. In the late seventies, Van der Gaag (1987) observed increased embryonal mortality and morphological abnormalities in fish exposed to Rhine water. Slooff (1982b) found no carcinogenic effect in Rhine fish but Vethaak and Rheinallt (1992) observed an increased incidence of hepatic tumors in flounders (Platichthys flesus) of the Dutch coastal zone of the North Sea.

4.3.3. Acute toxicity in the Daphnia assay

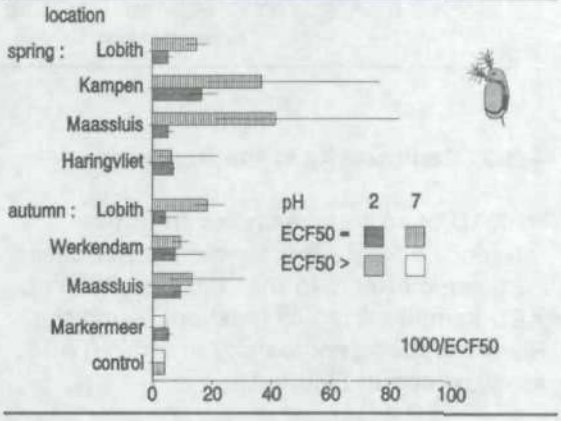
4.3.3.1. Control samples, reference site and differences between fractions

The XAD laboratory control samples did not induce response up to the tested maximum concentration factor of 250. The same was true for the pH 7 isolate from the sample of Markermeer, but a ECF50(2d) of 190 was calculated for the acid isolate of this reference site. The diethylether, ethylacetate, ethanol and ethanol:water fractions of all locations produced ECF50 values of 200 and above. As this is close to the level observed for the reference location and the laboratory control, we will not elaborate on these fractions but focus on the isolates and the cyclohexane fraction.

4.3.3.2. Toxicity in the Rhine Delta

In conformity with the sum of peak intensities, the acute toxicity of the autumn pH 7 samples at the downstream locations of Werkendam and Maassluis was lower than at the border location in Lobith, as shown in Figure 31. However, the Maassluis sample was more toxic than the Werkendam sample whereas the sum of peak intensities was higher at Werkendam than at Maassluis.

Figure 31. Average (with 95% c.i.) toxic response of Daphnia magna in short-term assays exposed to isolates from several locations. The highest 1000/ECF50 values were observed at the upstream locations in pH 7 isolates.



The ambient pH XAD-isolates from Maassluis and Kampen in the spring induced 50% response in D. magna at a twenty-five-fold concentration. The samples of Lobith and the September sample of Maassluis needed a more than fifty-fold concentration to induce the same effect. *The acute toxicity in the Werkendam and* Haringvliet samples was still twice as high as in the reference site. Additional toxicity was observed in the pH 2 XAD-isolates. This toxicity was always lower than in the corresponding pH 7 isolates, but it can contribute substantially to the total toxicity, as for instance in Kampen.

4.3.3.3. Trends in acute toxicity

Until 1977, D. magna exposed to Rhine water died within 2 days (Slooff 1983b) but the lowest ECF50 of 25 reported in this paper demonstrates that a significant improvement of water quality has been achieved over the past decade. At present, the range of contamination observed in the Rhine seems near that of other polluted rivers in Europe. For instance, a similar

range of acute toxicity was observed in extracts from the Po (Galassi et al. 1992) and from the Meuse (Maas-Diepeveen et al. 1992).

Comparable studies (Slooff et al. 1983, Slooff 1983a, De Zwart and Folkerts 1990) have been carried out in the past with guppies (Poecilia reticulata), several macrofauna species and luminescent bacteria (Photobacterium phosphoreum). In the early eighties, Rhine water had to be concentrated more than 100 times to cause mortality in guppies (Slooff et al. 1983) and in several macrofauna species, including Gammarus pulex (Slooff 1983a).

Recently, De Zwart and Folkerts (1990) reported ECF50 values ranging from 55 to 330 for luminescent bacteria (Photobacterium phosphoreum) exposed to Rhine water. This toxicity is about twice as low as we observed in D. magna in this study. It may be attributed either to differences in the XAD extraction techniques or to the specific sensitivity of the species. The XAD-extraction method used by De Zwart and Folkerts (1990) covers a smaller range of compounds than our method (Noordsij 1983, Noordsij 1985) but it is questionable here whether the extraction technique has influenced the outcome. Almost all the acute toxicity is recovered in the most lipophilic fraction and the performance of both XAD methods may be similar for this type of compounds. According to Munkittrick (1991) the sensitivity of P. phosphoreum and D. magna to organic microcontaminants is approximately equal. Yet, Maas-Diepeveen et al. (1991) found lower toxicity in P. phosphoreum than in D. magna assays with Meuse water.

De Zwart and Folkerts (1990) found a decrease of toxicity in a downstream direction to the Rotterdam harbour area, and some increase afterwards. Our autumn results, taken within a short time span, confirm this trend for total concentrations and Daphnia toxicity but not for the sum of peak intensities. Temporal and spatial variation of toxicity may be due to variables as emission rates, hydrographical conditions, river discharge, sediment load and plankton dynamics.

4.3.3.4. Most acute toxicity remains unidentified

For analysis of the compounds that may have caused the toxicity we focused on the cyclohexane fraction, which produced ECF50 (see Table 6.) similar to those of the isolates in Figure 31.

If the toxicity of a mixture can be described by concentration addition (see e.g. Könemann 1979), the ratios of exposure and median response concentrations per compound are expected to add up to one toxic unit (TU) as

Equation 1.

$$\sum_{1}^{n} \frac{C_i}{EC50_i} = \sum_{1}^{n} \frac{exposure\ concentration_i}{median\ response\ concentration_i} = 1\ TU$$

The appendix shows the apparent concentrations of the compounds that were identified in different samples. As discussed before, reduced isolation recovery and variability of response factors may influence the actual concentration present. The concentration in the assays to which the waterfleas are exposed at 50% response equals the product of the concentration in the water sample and the median response factor ECF50 of the assay.

Consulting the AQUIRE database (Pilli et al. 1989), containing information on short-term (1-4d) toxicity of 670 compounds to waterfleas (Cladocera), and the AQUATOX-database (BKH 1990) yielded data for 44 of the 158 compounds identified. For another 72 compounds, minimum EC50 values for D. magna were estimated from a QSAR (Quantitative Structure Activity Relationships) model (Montana State University 1989).

Application of the mixture model to the data in the appendix produced an estimated toxicity of 0.11 (=11%) TU at most. For each sample, about half of this toxicity could be attributed to the compound with the highest C_i:$EC50_i$ ratio.

The deviation from the expected 1 TU may be caused by several factors:

1. The assumption that isolation recovery of the identified compounds was similar to that of the reference compound may not be correct for all compounds.

Table 6. Minimum, maximum and geometric mean of acute response concentrations (EC50, LC50) for waterfleas (Cladocera) collected from the AQUIRE-database (Pilli et al. 1989).

	compounds	CAS number	EC50(1..4d)			n
			μ	min.	max.	
			$\mu g \cdot l^{-1}$			
biocides:	atrazine	1912249	$2*10^4$	$4*10^3$	$5*10^4$	6
	dichlobenil	1194656	$6*10^3$	$4*10^3$	$1*10^4$	6
	propazin	139402	$1*10^4$	$9*10^3$	$1*10^4$	2
	simazine	122349	$9*10^3$	$1*10^3$	$4*10^5$	7
hydrocarbons:	acenaphthene	83329	$3*10^4$	$3*10^3$	$3*10^5$	3
	anthracene	120127	$2*10^2$	$4*10^1$	$3*10^3$	3
	cycloheptane	291645	$5*10^4$	$5*10^4$	$5*10^4$	2
	ethylbenzene	100414	$2*10^4$	$2*10^3$	$2*10^5$	5
	methylnaphthalene	91576	$2*10^3$	$1*10^3$	$2*10^3$	3
	naphthalene	91203	$4*10^3$	$6*10^1$	$2*10^4$	14
	pyrene	129000	$2*10^2$	$9*10^1$	$2*10^3$	3
nitrogen compounds:	2,3 or 2,4 or 2,6-dinitrotoluene	606202	$2*10^4$	$2*10^4$	$2*10^4$	4
	aniline	62533	$5*10^2$	$1*10^2$	$2*10^4$	13
	dimethylaniline	95681	$3*10^4$	$3*10^4$	$3*10^4$	2
	nitrobenzene	98953	$3*10^4$	$2*10^4$	$6*10^4$	3
oxygen compounds:	1,2-benzenedicarboxylic acid, diethyl ester	84662	$6*10^4$	$5*10^4$	$8*10^4$	3
	1,2-benzenedicarboxylic acid, dimethylester	131113	$7*10^4$	$3*10^4$	$2*10^5$	2
	butylbenzylphthalate	85687	$2*10^4$	$2*10^3$	$5*10^5$	4
	cyclohexanone	108941	$8*10^5$	$8*10^5$	$8*10^5$	2
	di-iso-dibutylphtalate	84742	$4*10^3$	$4*10^3$	$5*10^3$	2
	phthalic acid, bis(2-ethylhexyl) ester	117817	$5*10^3$	$1*10^2$	$7*10^4$	3
phenols:	4,4'-isopropylidenediphenol	80057	$1*10^4$	$1*10^4$	$2*10^4$	2
	dimethylphenol	576261	$1*10^4$	$1*10^4$	$1*10^4$	6
	phenol	108952	$3*10^4$	$4*10^3$	$1*10^5$	68
halogen compounds:	3,4-dichloroaniline	95761	$1*10^3$	$2*10^2$	$2*10^4$	21

references: Abernethy et al. 1986, Adema 1978, Adema and Vink 1981, Alexander et al. 1988, Bailey and Liu 1980, Bobra et al. 1983, Bringmann and Kuhn 1977, Cairns et al. 1978, Calamari et al. 1980, Call et al. 1983, Canton and Adema 1978, Carlson and Caple 1975, Carlson et al. 1977, Cowgill et al. 1985, Crider et al. 1982, Crosby and Tucker 1966, Crossland and Hillaby 1985, Degraeve et al. 1980, Dowden and Bennett 1965, Eastmond et al. 1984, Ewell et al. 1986, Fizmayer et al. 1982, Frear and Boyd 1967, Galassi et al. 1988, Geiger et al. 1980, Geiger and Buikema 1982, Gersich and Mayes 1985, Gledhill et al. 1980, Hartman and Martin 1985, Hermens et al. 1984, Holcombe et al. 1987, Johnson and Finley 1980, Kamshilov and Flerov 1978, Keen and Baillod 1985, Kopperman et al. 1974, LeBlanc 1980, Lewis 1983, Liu et al. 1976, Macek et al. 1976, Marchini et al. 1988, McCarthy and Whitmore 1985, Millemann et al. 1984, Moore et al. 1987, Narbonne 1979, Passino and Smith 1987, Pearson et al. 1979, Randall and Knopp 1980, Sanders 1966, Sanders and Cope 1970, Semov and Iosifov 1973, Trabalka and Burch 1978, Trucco et al. 1983, Ziegenfuss et al. 1986.

2. Likewise, the assumption that the EC50 values collected from databases were measured in experimental conditions that were similar to ours may have been wrong. Yet, we selected fairly reliable data obtained under the test conditions that are, on average unlikely to deviate very much from the standard protocols we used. We did not use the minimum of the measured EC50 set as this is likely to reflect extreme conditions different from our experiments.
3. Next, the assumption that the XAD-extraction does not enhance the availability of the compounds for uptake by the waterfleas may have been violated. Comparing untreated with XAD-concentrated waste water Van der Gaag et al. (1990) for instance, found a slight increase possibly due to the

presence of a solvent that facilitates the uptake of the most lipophilic compounds. On the other hand, McCarthy (1989) concluded that (dissolved) humic substances decrease the availability of (lipophilic) organic microcontaminants, so that extracts are more likely to be less toxic in the presence of concentrated natural compounds. Our study did not yield any indication in this direction, as the effect in the total sample (with many humic acids) was of the same order of magnitude as in the cyclohexane extract *from this sample, in which humic acids* were almost absent (see Figure 25.).

4. The assumption of concentration addition in mixture may be wrong, indicating a synergistic behaviour of some compounds. Yet, laboratory experiments showed that the toxicity of mixtures containing organics with similar as well as with different modes of action (Hermens et al. 1984a, 1984b, Deneer et al. 1988) followed the concentration addition model.
5. The effects are caused by identified compounds of which we have no toxicity data. This suggests that our databases on toxicity assays are not adequate in regulating the discharge of compounds in aquatic ecosystems,
6. The effects are caused by substances that we cannot identify. This conclusion is evident for genotoxic compounds, which are present mainly in fractions that escape our classical analytical approaches. For the compounds causing acute toxic effects, identification should be possible here, as they are essentially found in the more lipophilic fractions.

To our opinion the last two factors are likely to contribute substantially to the observed difference between measured and expected toxicity, though we fully recognize that the importance of the other factors is, at least, not completely understood. Yet, irrespective of the actual cause of the deviation, application of chemical analysis alone would have led to underestimation of the toxicity of the samples.

4.3.4. Chronic toxicity and ecological significance

Exposure of D. magna to the XAD pH 7 isolate from the reference site Markermeer up to the highest concentration factor of 30 did not cause any mortality within 21 days. Survival and reproduction (in terms of LCF50, population-growth NOEC) was affected in XAD pH 7 isolates from the main river branches after about 5-10 times of concentration. The difference of one order of magnitude between acute and chronic toxicity is in accordance with *average acute-chronic ratios (e.g. Slooff et* al. 1986, Giesy and Graney 1988). Experiences with a biological early warning system at Lobith show that cohorts of D. magna survive in Rhine water for at least 1 week (Hendriks and Stouten 1992). At the end of this period they usually produce offspring. One may conclude that D. magna tolerates the average Rhine water quality, including the organic fraction isolated by XAD-resins.

Yet, these conclusions are limited to the response of our Daphnia magna strain to XAD-concentrated compounds and extrapolation to other compounds and species is limited. D. magna appeared to be several times less sensitive to some compounds than related Cladocera in semi-field experiments (Scholten, personal communication), whereas sensitivity even varied between different strains of D. magna (Baird et al. 1991).

Obviously, several groups of chemicals, including metals, very lipophilic and very hydrophilic organics are not extracted by XAD. Lipophilic substances with an octanol-water partition ratio K_{ow} higher than 10^5 are strongly bound to suspended solids and will therefore not adsorb effectively to XAD (Van der Gaag et al. 1990). Their significance is covered in accumulation studies carried out in the Rhine delta (e.g. Hendriks and Pieters 1993). Also, we could not evaluate the very hydrophilic compound that were present in the over 50% of the DOC not concentrated by XAD.

4.4. Conclusions and recommendations

We conclude that

1. Up to 50% of the organic compounds present in Rhine water can isolated with the XAD-extraction technique described in this paper.
2. *Over the past 10 years the toxicity of* Rhine water in terms of acute mortality in Daphnia magna has markedly improved but the genotoxicity, measured in the Ames test remained the same.
3. Nowadays, Rhine water induces acute mortality to Daphnia magna after a 25-fold XAD-concentration, whereas chronic toxicity occurs at lower concentration factors. Laboratory Daphnia magna seem to survive unconcentrated Rhine water well. It is recognized that more sensitive animals are likely to be found among other strains and species of cladocerans. The ecological significance of mutagenicity remains unknown.
4. Toxicity was exclusively observed in the cyclohexane fraction, whereas genotoxicity was mainly found in the *ethanol, ethylacetate and diethylether* fraction. This suggests that toxic compounds are of a rather lipophilic nature whereas mutagenic compounds are of a more hydrophilic kind.
5. The toxicity of the GC-MS identified compounds of the cyclohexane fraction was estimated using the concentration addition model and EC50 measurements collected from databases. The expected toxicity of the samples was 0.11 TU or less, leaving the rest to be attributed to lower recovery rates in the GC-MS analysis, differences in experimental conditions of toxicity assays, synergism and an increase of bioavailability after XAD-extraction. Yet, the difference is most likely to be caused by a lack of toxicological knowledge on identified compounds as well a on compounds that *were not identified by GC-MS.*
6. The conclusions only apply to compounds extracted by XAD. This study does not claim to cover metals, very lipophilic and very hydrophilic organics not concentrated by XAD.

Although major improvements have been achieved in the water quality of the Rhine over the past 10 years, the present situation still presents a threat to the ecosystem and reductions of emissions should continue. Considering the uncertainties about the toxicity of mixtures of largely unidentified compound present in surface water we conclude that the *technical means are insufficient to analyze* organic toxic pollutants or that toxicological information on identified compounds is lacking. In any case, application of toxicological assays for evaluation in of effluents and surface water will remain necessary. Fortunately, water quality managers of the Rhine river are more and more willing to introduce large-scale monitoring by both static and flow-through assays with organisms.

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Appendix

The compounds that were identified at several locations with the toxicity data used in the calculations described in the text.

			EC50	Marker-meer	Kampen	Lobith	Lobith	Werkendam	Maas-sluis	Maas-sluis	Haringvliet
sample date :				24.01	21.04	17.03	15.09	29.09	26.05	22.09	19.05
ECF50 :				250	24	80	84	283	26	126	106
		CAS					µg·l⁻¹				
Biocides:											
atrazine	1	1912249	$2*10^{4}$	0.035	0.074	0	0.025	0.054	0.096	0.029	0.104
dichlobenil	2	1194656	$6*10^{3}$	0.02	0	0	0	0	0.026	0.011	0
metolachlor	1	5121845	$3*10^{4a}$	0	0.018	0	0	0	0.089	0.024	0
n,n-diethyl-3-methyl-benz-amide	2			0	0	0	0.046	0.046	0.027	0.04	0.021
propazin	3	139402	$1*10^{4}$	0	0	0	0	0	0.015	0	0
simazine	2	122349	$9*10^{3}$	0	0.031	0	0	0.039	0.055	0.03	0.043
triadimefon	1	43121433		0	0	0	0.019	0	0	0	0
triadimemol	1			0	0	0	0.034	0.03	0.025	0.023	0
Hydrocarbons:											
1,1,3-trimethyl-2-methylene-indoline	3	84833		0	0	0	0.193	0	0	0	0
1,2-diphenylethane	2	103297	$2*10^{2q}$	0	0	0	0.049	0.02	0	0	0
1- or 2-methylnaphtalene	2	91576, 90120	$2*10^{3}$	0	0	0.066	0	0	0	0	0.023
2,3-dihydro-1h-indene	2	496117	$5*10^{3q}$	0	0	0	0	0	0.023	0	0
2,6-dimethyl-2,5-heptadiene-4-on	3	504201		0	0.03	0	0	0	0.06	0.079	0
2-ethyl-1-hexanol	3	104767	$1*10^{4q}$	0	0	0	0.025	0	0.026	0.011	0.023
4-tert-butylcyclohexanone	3	98533	$2*10^{4q}$	0	0	0	0.017	0	0	0	0
acenaphthene	2	83329	$3*10^{4}$	0	0	0.104	0	0.03	0	0	0.03
anthracene'	2	120127	$2*10^{2}$	0	0	0.026	0	0	0	0	0.021
bicyclo[2.2.1]heptane	3			0	0.093	0.095	0.034	0.052	0.072	0.057	0.081
cycloheptane	3	291645	$5*10^{4}$	0	0	0.027	0	0	0	0	0
ethylbenzene	2	100414	$2*10^{4}$	0	0	0	0	0	0.017	0	0
methylbifenyl → 3-methylbiphenyl	3	643936	$5*10^{2q}$	0	0	0.035	0	0	0	0	0
methylnaphtalene and nitrogen-compound → 1- or 2-methylnaphtalene	3	90120, 91576	$1*10^{3q}$	0	0	0	0.022	0	0	0.02	0.016
methylnaphthalene	2	91576	$2*10^{3}$	0	0	0.066	0	0	0	0	0.025
naphthalene	2	91203	$4*10^{3}$	0.025	0	0.054	0	0.026	0	0	0.021
pyrene	2	129000	$2*10^{2}$	0	0	0	0	0	0	0.026	0
miscellaneous :											
ferroceen	2	102545		0	0	0.037	0.042	0	0	0	0
n-ethyl-n-phenylformamide	3	5461494	$5*10^{4q}$	0	0	0	0.026	0.028	0	0.023	0
Nitrogen compounds :											
(2,4,6?)-trimethylpyridine	3	108758	$1*10^{4q}$	0	0	0.025	0.032	0.017	0	0	0
(2-nitro)-benzeneethanol	3	15121843	$6*10^{5q}$	0	0	0	0	0.017	0	0	0
1,2,3,3-tetramethylindoline → 1,2,3,3-tetramethyl-3h-indolium iodide	2	328979		0	0	0.078	0	0	0	0	0
1-ethoxy-2?-nitrobenzene → 1-ethoxy-2,4-dinitrobenzene	3	610548	$2*10^{3q}$	0	0	0	0.023	0.05	0	0.021	0
1-nicotine	2	54115	$9*10^{4q}$	0	0	0.032	0	0.022	0.02	0.026	0.019
2,2'-azobis-2-methylpropannitrill	2	78671	$9*10^{6q}$	0	0	0.072	0.055	0.041	0.033	0.045	0
2,3 or 2,4 or 2,6-dinitrotoluene → 2,6-dinitrotoluene	3	606202	$2*10^{4}$	0	0	0	0	0.039	0	0	0
2,3,3-trimethylindolenine	2	1640397		0	0.027	0	0.057	0	0.036	0.032	0
2,3,5-trimethylindol	2	21296924		0	0	0	0.035	0	0	0	0

			EC50	Marker-meer	Kampen	Lobith	Lobith	Werkendam	Maas-sluis	Maas-sluis	Haringvliet
sample date :				24.01	21.04	17.03	15.09	29.09	26.05	22.09	19.05
ECF50 :				250	24	80	84	283	26	126	106
	r	CAS					$\mu g \cdot l^{-1}$				
2,3-dimethylchinoxaline	2	2379557	$9*10^{4q}$	0	0	0	0.024	0	0	0.022	0
2?-methylchinoline	3	91634	$1*10^{4q}$	0	0.015	0	0	0	0.015	0.029	0.019
2?-nitrotoluene or 2-nitrobenzenethanol	3			0	0.017	0	0	0	0	0	0
2-chloropyridine	2	109091	$4*10^{4q}$	0	0	0	0	0.023	0	0	0
2-ethenylpyridine	2	100436	$3*10^{4q}$	0	0	0	0	0	0.03	0	0
2-methoxyaniline	2	90040	$5*10^{4q}$	0	0	0	0	0.023	0	0	0
2-methylpyridine	2	109068	$4*10^{4q}$	0	0.012	0.034	0	0	0.012	0.011	0
4-methyl-2-nitro-aniline	2	89623	$3*10^{3q}$	0	0	0.162	0.064	0.042	0.03	0.073	0.034
4-methylbenzenesulfonamide	3	70553	$2*10^{5q}$	0	0	0.063	0	0	0	0	0
4-picolylacetate	2			0.089	0.081	0.133	0.059	0.163	0.07	0.107	0.053
5-ethyl-2-methylpyridine[i]		104905	$1*10^{4q}$	0	0	0.046	0	0	0	0	0
aniline	2	62533	$5*10^{2}$	0	0.02	0.049	0.031	0.016	0	0	0.011
caffeine	2	58082		0	0.057	0.171	0.068	0.052	0.057	0.039	0.052
caprolactam	3	105602	$4*10^{5q}$	0	0	0.088	0	0	0	0	0
chinoline	2	91225	$3*10^{4}$	0	0	0.039	0.02	0.022	0	0	0.013
chloro-n,n-dimethylaniline → o-chloro-n,n-dimethylaniline	3	698011		0	0	0	0.098	0.14	0	0.033	0
dimethylaniline[i] → 2,4-dimethylaniline	2	95681	$3*10^{4}$	0	0	0.049	0.037	0.066	0	0.021	0
dimethylaniline[i] → 2,4-dimethylaniline	2	95681	$3*10^{4}$	0	0	0	0	0	0	0.024	0
dimethylchinoline	2			0	0.024	0	0.019	0.027	0.015	0	0
dimethylnitrobenzene → 1,5-dimethyl-2,4-dinitrobenzene	3	616728	$2*10^{4}$	0	0	0	0	0.023	0	0	0
dimethylpyridine[i]	2	108474	$2*10^{4q}$	0	0	0.029	0.029	0	0	0.01	0
ethylmethylpyridine[i] → 5-ethyl-2-methyl-pyridine	2	104905	$1*10^{4q}$	0	0	0	0	0.013	0	0	0
iminostilbene	3	256962		0	0	0.022	0.026	0	0	0	0
m- or p- methylpyridine → 3-methyl pyridine	2	108996	$4*10^{4q}$	0	0	0.038	0.021	0	0	0.018	0
m- or p-nitroaniline → p-nitroaniline	2	100016	$2*10^{4}$	0	0.057	0.261	0	0.063	0	0.018	0.036
methylnitroaniline → several methylnitro-anilines	2	99525, 99558, 89623, 119324	$1*10^{4q}$	0	0	0	0.023	0.034	0	0	0.013
n,n-dimethylaniline	2	121697	$5*10^{4q}$	0	0	0.146	0	0	0	0	0
n,n-dimethylbenzylamine	2	103833	$1*10^{5q}$	0	0	0.051	0.013	0	0	0.013	0
n,n-dipropyl-1-propaneamine	2	102692	$1*10^{4q}$	0	0	0.066	0.014	0.013	0	0	0
n-(2-methylphenyl)benzenesulfonamide	2	1845786		0	0.02	0	0	0.063	0	0.022	0
n-acetyl-n-ethylaniline	2	529657	$3*10^{5q}$	0	0.021	0.044	0	0.101	0.017	0.025	0.015
n-acetyl-p-methylaniline	2	103899	$3*10^{4q}$	0	0	0	0	0.038	0	0	0.035
n-butylbenzenesulfonamide	2	3622842	$4*10^{4q}$	0	0	0.087	0.039	0.079	0.048	0.038	0.046
n-cyclohexyl-cyclohexaneamine	3	101837	$3*10^{3q}$	0	0	0	0.015	0.025	0	0	0
n-ethyl-3-methylaniline	2	102272	$3*10^{4q}$	0	0	0	0.02	0	0	0	0
n-ethylaniline[i]	2	103695	$5*10^{4q}$	0	0	0	0	0.024	0	0	0
n-methylaniline	2	100618	$1*10^{5q}$	0	0	0	0	0.027	0	0	0
nitrobenzene	2	98953	$3*10^{4}$	0	0.028	0	0.022	0	0.035	0	0.024
o?-methoxynitrobenzene	3	100174	$7*10^{4q}$	0	0	0	0	0.039	0	0	0.011
o-chloroaniline	2	95512	$1*10^{4a}$	0	0	0.045	0.039	0.023	0.012	0.011	0
p,p'-bis(dimethylamino)benzofenon	3	90948	$3*10^{3q}$	0	0	0.035	0	0.021	0.025	0.021	0.019
propylaniline → 4-propylaniline	3	2696846	$7*10^{3q}$	0	0	0.036	0	0.094	0	0	0
tri-n-butylamine	2	102829	$6*10^{2q}$	0	0	0.035	0	0	0	0.016	0

			EC50	Marker-meer	Kampen	Lobith	Lobith	Werkendam	Maassluis	Maassluis	Haringvliet
sample date :				24.01	21.04	17.03	15.09	29.09	26.05	22.09	19.05
ECF50 :				250	24	80	84	283	26	126	106
		CAS		$\mu g \cdot l^{-1}$							
Oxygen compounds :											
α-methyl-propyl-benzeneethanol	2	54518115		0	0	0.026	0	0.021	0	0	0
α-methyl-propyl-benzeneethanol	2			0	0.016	0	0	0	0	0	0
α-methyl-propyl-benzeneethanol	2			0	0.015	0	0.018	0	0.018	0.018	0
1,2-benzenedicarboxylic acid, diethyl ester	2	84662	$6*10^{4}$	0	0	0	0.058	0	0.027	0.052	0.046
1,2-benzenedicarboxylic acid, dimethyl ester	2	131113	$7*10^{4}$	0	0.012	0.068	0.039	0	0.036	0.047	0.074
1,3-dimethoxybenzene	2	151100	$6*10^{4q}$	0	0	0	0	0.063	0	0	0
1-(2-methoxy-1-methylethoxy)-2-propanol	3			0	0	0.059	0.034	0	0.036	0	0
1-(2-propenyloxy)-2-propanol	3	21460366		0	0	0	0	0	0.022	0	0
2,3-dichloro-α-methylbenzyl alcohol	2	54798913		0	0.017	0	0.019	0	0.037	0	0
2,4,8,10-tetraoxaspiro 5.5undecane	3	126545		0	0	0	0	0.021	0	0	0
2,5,8,11,14-pentaoxapentadecane	3	143248	$6*10^{6q}$	0	0.041	0	0	0.078	0	0	0
2,5,8,11,14-pentaoxapentadecane	3	143248	$6*10^{3q}$	0	0.041	0	0	0.078	0	0	0
2,5-dihydro-2,5-dimethoxyfuran	2	332774		0	0	0.02	0	0	0	0	0
2-butoxyethanol	3	111762	$2*10^{6}$	0	0	0	0.036	0	0	0	0
3,5,5-trimethyl-2-cyclohexen-1-ol	2	470995	$4*10^{4q}$	0	0	0.032	0.037	0	0	0	0.012
3-hexanon	3	589388	$2*10^{5q}$	0	0	0.049	0	0	0	0	0
5-methyl-2-heptanon	3	18217124		0	0	0	0.045	0	0	0	0
acetofenon	2	98862	$1*10^{5q}$	0	0	0	0.029	0	0.015	0.016	0
benzaldehyde	2	100527	$5*10^{4}$	0	0.016	0	0.017	0	0.014	0.015	0.013
benzophenone	2	119619	$8*10^{3q}$	0	0	0	0	0	0	0	0.028
butylbenzylphthalate	2	85687	$2*10^{4}$	0	0	0.043	0.06	0	0	0.022	0
cholesterol	2	57885		0	0	0	0	0	0	0	0.123
cyclohexanol	2	928949		0	0.174	0	0.512	0.109	0.241	0	0
cyclohexanone	2	108941	$8*10^{5a}$	0	0	0.179	0	0.219	0.037	0	0.433
di-iso-dibutylphtalate → 1,2-benzenedicarboxylic acid, dibutyl ester	3	84742	$4*10^{3}$	0	0.049	0.091	0.141	0.048	0.067	0.041	0.069
di-iso-dibutylphtalate → 1,2-benzenedicarboxylic acid, dibutyl ester	3	84742	$4*10^{3}$	0	0.044	0.076	0.101	0.046	0.04	0.046	0.049
diacetonsorbose	1			0.208	0.272	0.478	0.165	0.324	0.285	0.203	0.18
ethanol-2-(2-butoxyethoxy)-acetate	2	124174	$4*10^{4q}$	0	0	0	0	0	0.031	0	0
hexanal	3	66251	$1*10^{4q}$	0	0.035	0	0	0	0.054	0.036	0.05
iso-octyldiisobutyrate	2			0	0	0	0.108	0	0.024	0	0
methyloroselol	3			0	0.035	0.054	0.03	0.028	0	0.019	0.021
phthalic acid, bis(2-ethylhexyl) ester	3	117817	$5*10^{3}$	0.012	0.04	0.063	0.036	0.022	0.024	0.023	0.035
propane acid, 2-methyl-3-hydroxy-2,4,4-trimmethylpentyl ester	2	74367343		0	0.042	0	0	0	0.033	0	0
tert-butylcyclohexenone	2		$2*10^{4q}$	0	0.023	0.04	0.03	0.017	0.044	0.042	0.033
tetraethyleneglycol, dimethylether	3	143248	$6*10^{6q}$	0	0.031	0.074	0.047	0.099	0.031	0.031	0.023
Phenols :											
2,6-di(tert-butyl)phenol	2	128392	$5*10^{2q}$	0	0	0.053	0.043	0.027	0	0.028	0
2-tert-butylphenol	3	88186	$4*10^{3q}$	0	0	0	0.022	0	0	0.015	0
4,4'-isopropylidenediphenol	2	80057	$1*10^{4}$	0	0	0.119	0	0	0	0	0
4-(1-methyl-1-fenylethyl)phenol	3	599644	$2*10^{3q}$	0	0	0.053	0	0	0.031	0	0.016

			EC50	Marker-meer	Kampen	Lobith	Lobith	Werkendam	Maas-sluis	Maas-sluis	Haringvliet
sample date :				24.01	21.04	17.03	15.09	29.09	26.05	22.09	19.05
ECF50 :				250	24	80	84	283	26	126	106
	[r]	CAS					µg·l⁻¹				
chloromethylphenol	2	6640273, 615747, 1570645, 59507	8*10[3q]	0	0	0.136	0	0	0	0	0
di-tert-butylcresol → 2,6-di-tert-butyl-p-cresol	3	128370	1*10[3]	0	0	0.056	0	0	0	0	0
di-tert-butylphenol → 4-tert-butylphenol, acetate	3	3056642		0	0.048	0.071	0.058	0	0	0	0
dibutyl-phenol	3			0	0	0.09	0	0	0	0	0
dimethylphenol → 2,6-dimethylphenol	3	576261	1*10[4]	0	0	0	0.03	0	0.018	0	0
dimethylphenol[i] → 2,6-dimethylphenol	2	576261	1*10[4]	0	0	0	0.017	0	0	0.008	0
dimethylphenol[i] → 2,6-dimethylphenol	2	576261	1*10[4]	0	0	0.024	0	0.015	0	0	0
o-, m-, or p-tert-butylphenol → 3-(1,1-dimethylethyl)phenol	2	585342	4*10[3q]	0	0.022	0.048	0.014	0	0	0	0
phenol	2	108952	3*10[4]	0	0	0	0.026	0	0	0	0
phenylphenol	2	90437	7*10[3q]	0	0	0.06	0.039	0.033	0	0	0
tert-butylcresol	2			0	0.057	0.157	0	0	0	0	0
trimethylphenol → 2,4,6-trimethylphenol	2	527606	4*10[3q]	0	0	0	0.023	0	0	0	0
Sulphur and phosphor compounds :											
1-(2-benzothiazolyl)-1,3-dimethylurea	1	18691979	3*10[4s]	0	0.024	0	0	0.021	0	0	0
2-(methylthio)benzothiazol	2	615225	1*10[4q]	0	0.027	0.071	0.063	0.039	0.03	0	0.029
benzothiazol	1	95169	5*10[4q]	0	0	0	0.023	0	0	0.022	0.024
chloro(methylsulfonyl)benzene[i]	2	98577	6*10[5q]	0	0	0	0.151	0	0	0.064	0
chloromethylsulfonylbenzenes[i]	2	98577	6*10[5q]	0	0	0	0.039	0	0	0.036	0
mixture with triethyl phosphate → triethyl phosphate	3	78400	1*10[5]	0	0.033	0	0	0	0.048	0	0.031
mixture with triethyl phosphate → triethyl phosphate	3	78400	1*10[5]	0	0	0	0	0.085	0	0.116	0.031
diphenylsulfone	2	127639	5*10[4q]	0	0	0	0.032	0.035	0	0.023	0
tri(ethanol-2-butoxy)phosphate	2	78513	2*10[4q]	0	0.115	0.259	0	0	0.089	0.077	0.084
tri(tert-butyl)phosphate	2	126716	1*10[4q]	0	0.069	0.198	0.071	0.08	0.096	0.049	0.05
triethyl phosphate	2	78400	1*10[5]	0.079	0.042	0	0.077	0	0.422	0.079	0.05
trimethylthiophosphate	3			0	0	0.036	0	0	0	0	0.011
triphenylphosphine oxide	2	791286	3*10[4q]	0	0.046	0.195	0.139	0.089	0.089	0.108	0.103
triphenylphosphine sulfide	2	3878453		0	0	0	0.029	0	0	0	0
1,2,3-trichloropropane	2	96184	1*10[5q]	0	0	0	0	0	0.066	0.027	0
1-chloro-2-nitrobenzene	2	88733	5*10[4q]	0	0	0	0.015	0	0.027	0.023	0.018
1-chloro-4-nitrobenzene[i]	2	100005	3*10[4q]	0	0	0.063	0	0.055	0	0	0
bis(dichloro-n-propyl)ether	2			0	0	0	0	0	0.015	0	0
chloroether (C3Cl2-O-C6Cl3)	3			0	0	0	0	0	0.04	0	0
chloroether (C3Cl2-O-C6Cl3)	3			0	0	0	0	0	0.021	0	0
chloroether (C3Cl2-O-C6Cl3)	3			0	0	0	0	0	0.032	0	0
cyclohexyliodide	2	626620	3*10[3q]	0.011	0	0	0	0.017	0	0.007	0
dichloroaniline[i] → 3,4-dichloroaniline	2	95761	1*10[3]	0	0	0	0	0.043	0	0	0
dichloroaniline[i] → 3,4-dichloroaniline	2	95761	1*10[3]	0	0	0	0.039	0	0	0	0
dichloroaniline[i] → 3,4-dichloroaniline	2	95761	1*10[3]	0	0	0.027	0.026	0	0	0.018	0
tri(chloropropyl)phosphate → tris(dichloropropyl)phosphate	3	13674878	4*10[5q]	0	0	0	0	0.055	0	0	0
tri(chloropropyl)phosphate → tris(dichloropropyl)phosphate	3	13674878	4*10[5q]	0	0	0	0	0.038	0.024	0.026	0.025

			EC50	Marker-meer	Kampen	Lobith	Lobith	Werkendam	Maassluis	Maassluis	Haringvliet
sample date :				24.01	21.04	17.03	15.09	29.09	26.05	22.09	19.05
ECF50 :				250	24	80	84	283	26	126	106
		CAS					$\mu g \cdot l^{-1}$				
tris(ß-chloroethyl)phosphate	2	115968		0.05	0.056	0.129	0.056	0.172	0.067	0.092	0.048
n concentration in water sample * ECF50				[c]0.38%	0.18 %	4.28%	3.50%	6.63%	0.18 %	3.10 %	1.16%
Σ EC50			=								
1				[a]0.45%	0.28 %	7.72%	5.22%	11.3%	0.35 %	4.31 %	3.34%

[i]identification reliability: 1 = complete resemblance of retention time and mass spectrum with that of the pure compound, 2 = resemblance of the mass spectrum with that of an external library, with addition of expert judgment, 3 = less certain identification, → = CAS and toxicity refer to specific more or less similar compound, [i]isomere
toxicity data from AQUIRE-database (see Table 6., except [a]AQUATOX measurement or [q]QSAR-estimation
toxic units were estimated with sets containing [a]all (partly) identified compounds and [c]completely identified compounds only. For the last set the highest contribution to the toxicity were attributed to naphthalene, (0.15%), aniline (0.10%), 2,6-di(tert-butyl)phenol (0.79%), 1,2-diphenylethane (1.68%), 1,2-diphenylethane (2.29%), 4-methyl-2-nitro-aniline (0.02%), pyrene (1.34%), aniline (0.23%) in the samples from the subsequent locations.

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5. MONITORING THE RESPONSE OF MICROCONTAMINANTS BY DYNAMIC DAPHNIA MAGNA AND LEUCISCUS IDUS ASSAYS IN THE RHINE DELTA: BIOLOGICAL EARLY WARNING AS A USEFUL SUPPLEMENT

A. JAN HENDRIKS[1], MINOU D.A. STOUTEN[2]

[1]Institute for Inland Water Management and Waste Water Treatment, RIZA, P.O. Box 17, 8200 AA, Lelystad,
[2]BKH, Consulting Engineers, P.O. Box 5094, 2600 GB Delft
The Netherlands

Abstract

Following some severe upstream episodes in the eighties, biological early warning systems with fish and waterfleas were installed at monitoring stations in the Rhine and the Meuse. Laboratory tests suggest that these whole-organism monitors are likely to respond at concentrations close to lethal levels. Current average and peak concentrations of compounds that can be identified and of which information on toxicity is available are often below these levels. Nevertheless, several alerts were registered in recent years. This may be attributed to the combined effect of known and unknown compounds under prevailing field conditions. Results are compared to experiences at other locations and some prospects on development in the future are given.

Keywords: biological early warning, continuous monitor, Daphnia magna, Leuciscus idus, Rhine, flow-through, Rhine, dynamic assays

5.1. Introduction

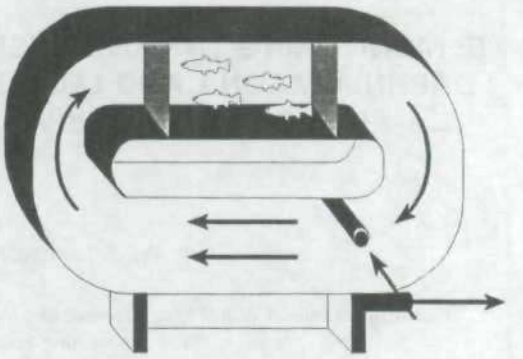
A major episode in 1986, when the runoff from a fire in the Sandoz plant at Basel wiped out part of the wildlife in the river, revived the concern about the Rhine water quality. It stimulated further international cooperation, which led to the setup of the "Rhine Action Program". The aim of this program is to achieve a further reduction of the pollution and to restore destroyed habitats, thus enabling the return of indigenous species (IRC 1987).

In The Netherlands, chemical control of river water entering at the border locations was extended. Yet, it was recognized that chemical warning cannot detect all of the thousands of compounds present in the Rhine at a reasonable cost and introduction of biological early warning systems was considered necessary. The systems may not only help to detect increased levels of compounds that cannot be detected chemically but also give an impression of the toxicity of all compounds under prevailing field conditions. Determinants as temperature, pH, hardness, oxygen pressure, dissolved and particulate organic carbon levels, salinity and concentrations of other pollutants can have a large impact on the actual damage caused by a microcontaminant.

In the past, many biological early warning systems have been developed for monitoring water quality. Though often excellent in laboratory, only a few have survived the harsh conditions of field application. In the seventies, Rhine water was monitored by a fish system by Poels (1977) but the system was abandoned because of the high suspended solid loads. Since then, new systems were designed, and 1 decade later the Institute of Inland Water Management and Waste Water Treatment decided to test several systems for application in the routine program. In their review, Kramer and Botterweg (1991) concluded that only a few systems were commercially available and two of these, one with fish and one with waterfleas, were selected. The systems were installed successively at the monitoring stations in

Figure 32. The Rhine delta with surface- and drinking water control stations equipped with a early warning fish monitor. In addition, waterflea monitors are located at Lobith and Eijsden.

Figure 33. The dynamic fish (Leuciscus idus) assay by Juhnke and Besch (1971).



the Rhine and the Meuse, located at the border with Germany and Belgium respectively, as illustrated by Figure 32. At present, fish early warning systems are implemented by a routine monitoring program, while dynamic waterflea assays are still being tested.

5.2. Methods

5.2.1. Dynamic fish assays

The first version of the fish monitor used was designed by Juhnke and Besch (1971). The heart of the system, as illustrated in Figure 32., consists of a flow-through basin. It contains 4 individuals of a gold-coloured variety of the indigenous ide, Leuciscus idus, a member of the carp family, Cyprinidae. Fish were obtained from a fish farm, and judged for good health and a minimum length of 12 cm before use. During 2 minutes in a 10 minute cycle, water flows through the basin at 30 $l \cdot min^{-1}$. It forces fish to swim upstream in conditions similar to those of outdoor running waters.

The back of the basin is separated from the rest of the system by bars. Changes in air pressure, induced by fish touching these bars, are registered by a microphone. The response is recorded on disk but behavior is regularly observed by eye too. Occasionally, fish strike against the sensor, causing a background level of impulses. If fish are weakened or try to avoid the incoming water flow, the number of impulses increases.

To obtain a qualitative and quantitative impression of the response, several laboratory tests were carried out. After 1 day of acclimatization, a single dose was added to the recycling water, thereby generating a step-like exposure concentration in time. The lowest concentration that induced impulses after exposure of t days, EC(t) is reported.

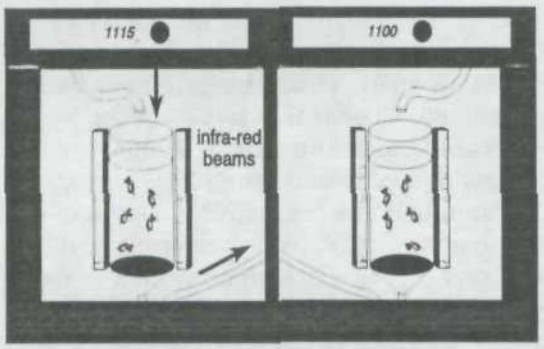
In field applications fish are exposed continuously for a 1 week period. In the first 6 to 12 hr the number of touches is somewhat higher than in the rest of the week. The alert level was set on the maximum number of impulses observed in these acclimatization periods after several months of application. The systems are connected to a monitoring network so that its response can be compared to other on-line variables like temperature, pH, oxygen contents and heavy metal concentrations. If the alert level is exceeded, water samples are taken automatically and submitted to analysis by gas chromatography and mass spectrometry and, since 1990, to static assays.

5.2.2. Dynamic waterflea assays

The dynamic waterflea assay used was designed by Knie (1978, 1982) and is illustrated by Figure 34. It has a pair of 0.03 l chambers that contain waterfleas and through which water flows at a rate of 0.5 $l \cdot h^{-1}$.

At both sides of each chamber, infra-red sources and receptors register waterfleas

Figure 34. The dynamic waterflea (Daphnia magna) assay. Activity is registered as the number of infrared beam interruptions per 10 min.



crossing the lightbeams. The swimming activity is recorded as the number of interruptions per 10 min.

For tests executed in our laboratory 20 juveniles of ≤2 d were put into the system at a temperature of 20±1°C. After several hours of acclimatization, the compound was added as described for the fish monitor. The lowest concentration EC(t) that induced a decrease of activity is reported.

In field tests at the monitoring station, 20 juvenile waterfleas are exposed to Rhine water. To avoid clogging of small tubes, large suspended solids are filtered out of the sample water but small organic particles flows through to feed the daphnids. To reduce maintenance, the same cohort of organisms was monitored for 7 days. In summer, juveniles are sometimes born before the end of the week and this disturbs the response pattern. Therefore the temperature was reduced to a level of 17±1°C, still high enough for waterfleas to survive and reproduce well. Unfortunately, regulation of temperature caused new problems, such as the emergence of air bubbles.

Activity increases as daphnids grow and at the end of the week young may be born. An absolute alert level is set on a fixed minimum. To register small deviations from the weekly trend, a flexible limit was developed. A response is considered an alert if the deviation of the normal activity exceeds a certain confidence level.

5.2.3. Static fish and waterflea assays

The median lethal LC50(t) (for fish) and sublethal EC50(t) (for waterfleas) values for static assays presented in this paper were extracted from two databases (Pilli et al. 1989, BKH 1990). As biological warning focuses on registration of short-term peaks all records with an exposure period of t≤5 d. To obtain a substantial set of information, the data were not confined to D. magna and L. idus but extended to all waterflea species, Cladocera, and the carp family, Cyprinidae. All data were first normalized to 1 day exposure, expressed as EC50(1) or LC50(1), assuming that the product of concentration and time is constant. Finally, we calculated the geometric average per compound for Cladocera and Cyprinidae thereby lumping together differences among species, lifestages and experimental conditions.

5.3. Results

5.3.1. General

One may judge the usefulness on characteristics that can be reduced to four properties. *Sensitivity* usually refers to the time and concentration required to induce an alert in laboratory tests and field surveillance. *Specificity* may be regarded as the extent to which the system responds, as intended, to microcontaminants versus the reaction to other stimuli. An impression may be obtained from the number of true and false alerts. The *significance* of a response for the ultimate objectives set, usually protection of the aquatic community is sometimes overlooked during development. To get some idea, one may compare the lowest response concentration EC(t) of a dynamic assay to EC50(1) and LC50(1) values for static assays. The success of monitors also depends on the *support* required. The fish and waterflea systems require about half a day of maintenance per week each. Regular inspection by eye of the systems is necessary and indispensable in case of alarms.

Figure 36. Total water and Cyprinidae LC50(1) concentrations of compounds monitored during episodes at Lobith (Schäfer and Breukel 1990, Pilli et al. 1989, B.K.H. 1990).

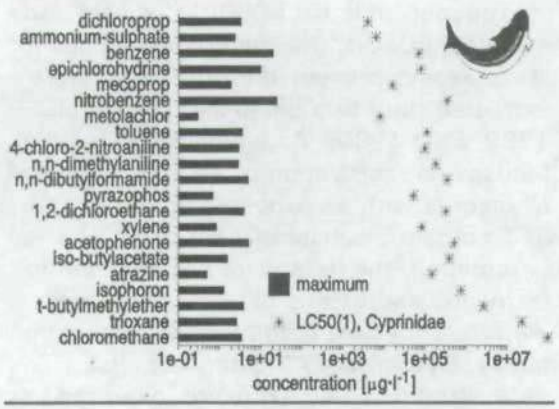


Table 10. gives the number of episodes detected by chemical and biological early warning. Though most upstream accidents are reported by the German authorities some increased concentrations were first detected by the early warning systems on the Rhine at Lobith. There, the number of episodes detected by chemical and biological early warning is in the same order of magnitude. In contrast, upstream episodes on the Meuse are seldom registered by Belgian authorities, so that early warning is even more necessary there.

Table 10. Number of increased concentrations at bordering monitoring stations: total, first detected by chemical and biological early warning.

episodes	Rhine					Meuse				
year	87	88	89	90	91	87	88	89	90	91
total	10	10	12	12	8	7	9	6	6	8
chemical warning	1	1	4	1		6	9	6	4	
biological warning	[a]	3	4	3	[p]	[a]	[a]	[a]	[p]	2
reported spills	54	23	60	30		1	0	0	2	

After two biological warnings no compounds were found (see Table 10.). Increased concentrations not first detected by biological or chemical warning were measured after reports of accidents by German and Belgium authorities. [a]absent, [p]partially present, chemical data from 1990 and 1991 not official.

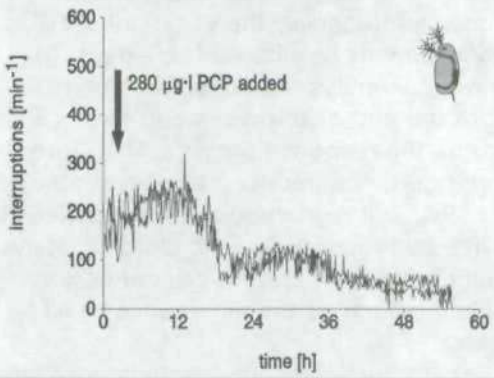
Upstream and downstream from Lobith, similar dynamic fish and waterflea assays have been implemented to monitor Rhine water quality. In 1991, one alert was registered on a fish monitor in the nearby German region Nord-Rhein Westfalen (Von Danwitz et al. 1992). In the same year, fish monitors at water intake locations downstream from Lobith did not register episodes

The fish monitor in the Meuse was implemented at Eijsden in the second half of 1990. In 1991, two fish alerts have been registered. Chemical analysis yielded increased concentrations of 7 $\mu g \cdot l^{-1}$ tributylphospate and 18 $\mu g \cdot l^{-1}$ trichloromethane respectively. Downstream fish monitors at drinking water inlets did not respond. In the Aa, a small stream in the north of The Netherlands, a fish early warning system frequently produces alerts (Zandberg, personal communication). This might be attributed to its close proximity to agricultural activities in the basin.

5.3.4. Laboratory experiments with waterfleas

Figure 37. is a typical example of the response of the waterflea monitor observed during tests in the laboratory.

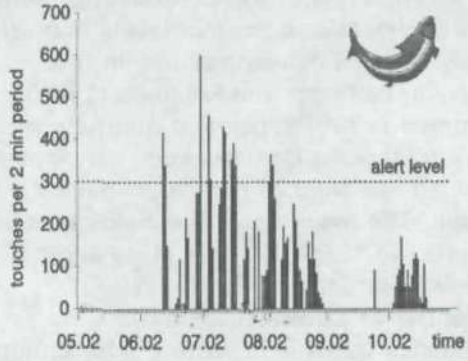
Figure 37. Typical response of the waterflea (D. magna) monitor in a laboratory test, here with 280 $\mu g \cdot l^{-1}$ pentachlorophenol. The response is registered as the number of infra-red beam interruptions.



After 12 h the activity decreases about 50%. Results of laboratory tests with other microcontaminants are summarized in Table 11.

One may conclude that differences between response concentrations of the flow-through and static assays are less than 5. In laboratory tests with 12 metals static EC50 values were at most 3 times as high as dynamic EC50 levels (Knie 1982). However, Knie (1988) estimated larger differences for some organic

Figure 35. Typical alert of the fish (L. idus) monitor, registered at Lobith in February 1989. The response is registered as the number of impulses generated by fish that touch the sensor.



Concentrations of compounds in the sample that were increased compared to average levels are also given. One may conclude that some polycyclic aromatic hydrocarbons occurred in concentrations that are close to the LC50(1). Yet, these lethal levels are derived from a few records present in the database (Pilli et al. 1989) so that (a combination of) other compounds may have contributed as well. The other compounds analyzed are less likely to be responsible because concentrations are generally more than 10,000 times below the LC50(1) values. Nevertheless, these microcontaminants cannot be excluded completely because species or end points may be specifically sensitive to the compounds analyzed.

The past 2 years, the number of alerts seem to have decreased. This coincides with a reduction of the number and the seriousness of reported episodes, as summarized in Table 10. In addition, increased pollution levels might have been missed due to maintenance problems. The sensitivity of the sensor may have decreased temporarily, partly caused by heavy suspended solid loads, as strong escaping behavior without alerts was observed on some occasions. As a result, vulnerable components of the system have been replaced and a method for calibration is being developed.

Half the episodes summarized in Table 10., refer to compounds of which data on fish toxicity could be obtained from databases (Pilli et al. 1989, BKH 1990). In Figure 36. the maximum concentrations measured at the Lobith monitoring station are compared with geometric means of LC50(1) for fish, largely Cyprinidae.

As the maximum concentrations are far below lethal levels, it will be of no surprise that these episodes did not provoke an alert in the dynamic fish assay.

Table 9. Dynamic fish (L. idus) response registered at the Rhine monitoring station of Lobith with the maximum Rhine water concentration (max(C)) and the median lethal response concentration LC50(1) of the compounds found.

date	compound	max(C)	LC50(1)
		$\mu g \cdot l^{-1}$	
15.04.88	isophoron	26	800000
06.07.88	isophoron	3	800000
26.10.88	fluoranthene	0.4	≈16000
	pyrene	0.3	≈10
	tetrachloroethane	10	≈50000
	tetrachloroethylene	0.2	≈40000
06.02.89	fluoranthene	0.5	≈16000
	pyrene	0.4	≈10
	trioxane	2	20000000
23.05.89	no compounds found at increased concentrations		
08.06.89	1,1,2,2-tetrachloroethane	2	60000
	1,1,2-trichloroethane	1	200000
	2-nitroaniline	3.1	
	atrazine	0.5	100000
	metolachlor	0.1	≈8000
	pyrazophos	0.2	50000
	simazine	0.2	200000
31.10.89	anthracene	0.4	≈40
	fluoranthene	2.0	≈16000
	fluorene	0.3	≈3000
	penconazol	0.3	
	phenanthrene	0.9	≈200
	pyrene	1.5	≈10
03.01.90	atrazine	0.1	100000
	pyrazophos	0.2	50000
	triphenylphosphineoxide	0.2	200000
08.02.90	2-nitroaniline	1.1	
	benzothiazole	0.3	
	hexanol-like compound	9.8	
	triphenylphosphineoxide	0.6	200000
16.06.90	no compounds found at increased concentrations		

5.3.2. Laboratory experiments with fish

Table 8. Dynamic L. idus and static Cyprinidae response concentrations and Dutch water quality standards.

compound	dynamic EC(t)		static[a] LC50(1)	stan-dard[b]
	$\mu g \cdot l^{-1}$	d	$\mu g \cdot l^{-1}$	
3,4-dichloroaniline	500	0.8	20000	
ammonia	1200	0.1	28000	20
cadmium	300	0.8	680	0.2
disulfoton	7000	0.8	10000	1.5
endosulfan	5[c]	1	6	0.01
endrin	15[c]	1	12	
linear alkyl benzene sulfonate	5400	3.2	7500	

[a]Pilli et al. 1989, B.K.H. 1990, [b]Stortelder et al. 1989, [c]Ermisch and Juhnke 1973.

Table 8. reflects concentrations in laboratory tests at which the dynamic fish assay responded within 1 day, with exception of a 3.2-d exposure period for linear alkyl benzene sulfonate. Responses may be expected earlier at higher concentrations and later at lower concentrations, probably with an almost constant product of exposure concentration and time, as indicated for endosulfan and endrin by Ermisch and Juhnke (1973).

One may conclude that alerts for the test compounds occur at nominal concentrations EC(t) of over 10 $\mu g \cdot l^{-1}$, which may be up to 40 times below the static LC50(1) for Cyprinidae. These differences may be partially caused by variability of species and experimental differences. For instance, Juhnke and Lüdemann (1978) report LC50 values of 8800 and 70000 $\mu g \cdot l^{-1}$ derived from static assays with L. idus at two different laboratories.

Alert levels of the tested compounds are also well above current water quality standards derived from no observed response concentrations for algae, crustaceans and fish (Stortelder et al. 1989).

Though the number of compounds tested is small, the ratios between responses in dynamic and static assays agree with conclusions from literature reviews. Baldwin (1990) and Little and Finger (1990) concluded that minor deviations in movements of fish may be observed at 1% of the LC50(acute). Serious loss of rheotaxis occurs at levels that are close to lethal concentrations. In their reviews, Beitinger and Freeman (1983) and Beitinger (1990) concluded that fish, mainly Salmonidae and Centrachidae, consistently avoided one-third of the 75 chemicals tested. The majority of thresholds was in the 100 to 1000 $\mu g \cdot l^{-1}$ range. In another review, Giattina and Garton (1983) collected 11 LC50(acute) levels and avoidance thresholds. With the exception of nickel, ratios were smaller than 70. More in general, Baldwin (1990) argued that responses of fish early warning are to be expected at 10% of the LC50(acute) and higher. As illustrated in Figure 39., most acute median lethal concentrations are over 10 to 100 $\mu g \cdot l^{-1}$, so one might expect detection limits of over 1 to 10 $\mu g \cdot l^{-1}$.

As far as specificity is concerned, the fish system is rather robust and does not seem to respond to changes in water quality that are not induced by microcontaminants. It is also less sensitive to other disturbances. From the laboratory results, one may conclude that the monitor is likely to respond to specific groups of microcontaminants that are toxic to fish in static assays with a short exposure period. As argued by Koeman et al. (1978), continuous biomonitoring systems, in particular with fish, will respond quickly to compounds that act on ventilation (phenols, organic solvents, ammonia, metals), respiration (nitro-, amino- and nitril-like compounds, cyanide, sulfides) and on the nervous system (some persistent chlorobiocides, pyrethroids, methylmercury).

5.3.3. Field surveillance with fish

Figure 35. gives the response of the dynamic fish assay observed at Lobith in February 1989.

As can be read in Table 9., increased concentrations of fluoranthene, pyrene and trioxane have been measured. Similar alerts that have been registered so far are also summarized in Table 9.

Table 11. Dynamic D. magna and static Cladocera response concentrations and Dutch water quality standards

compound	dynamic EC(t)		static EC50(1)	stan-dard
	$\mu g \cdot l^{-1}$	d	$\mu g \cdot l^{-1}$	
1,2-dichloroethane	100000[a]		360000	
3,4-dichloroaniline	3600	1.1	700	
cadmium	360	0.4	130	0.2
chlorophenol	20000[a]		≈10000	
diazinon	8[b]	0.3	2	
disulfoton	100[b]	0.5	<60	1.5
endosulfan	400[b]	0.5	510	0.01
etrimfos	4[b]		4	
linear alkyl benzene sulfonate	480	0.1	7800	
paraquat	>74000	0.3	4800	
parathion-ethyl	5[b]	0.1	2	0.02
parathion-methyl	5[a]	0.2	4	0.2
pentachlorophenol	280	0.6	1100	0.05
propethamphos	100[b]	0.5		
thiomethon	400[a]	0.5	<8200	

[a]Caspers (1988), [b]Matthias and Putzicha (1990), [c]Pilli et al. (1989), B.K.H. (1990), [d]Stortelder et al. (1989)

microcontaminants in Rhine water.

In general, we may conclude that the sensitivity and specificity of the waterflea monitor can probably be judged well by studying static EC50(acute) data. If true, one may expect dynamic waterflea assays *to be especially useful for compounds that* damage arthropods at low concentrations, such as many insecticides. This is confirmed by Von Danwitz et al. (1992) who reports waterflea monitor responses to organophosphor insecticides near the 1 $\mu g \cdot l^{-1}$ level.

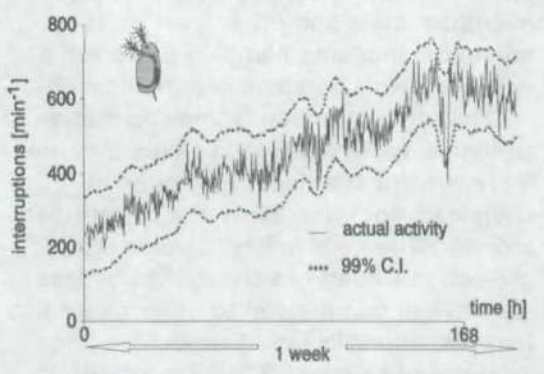
As it is often hard to discriminate between immobility and mortality in short term static assays with Cladocera (ISO 1989), differences between sublethal EC50(1) and lethal LC50(1) levels will be small. In another paper, Hendriks (1994) concluded that short term Cladocera assays for 123 compounds extracted from a database (Pilli et al. 1989) yielded LC50(1):EC50(1) ratios that were as frequently higher as lower than 1. In short-term static assays with concentrated Rhine water (Hendriks et al. 1994), mortality was generally observed at concentration factors that were 1 to 3 times higher than those at which immobility was observed first (Maas, personal communication). In the next lower dilution step no short-term response was visible. The reduction of swimming ability as recorded in dynamic assays may be conceived to precede immobility and mortality which are counted in static assays, but this has, to our knowledge, not been investigated quantitatively.

The dynamic experiments with the test compounds and small difference between no response and lethal concentrations in short-term static assays indicate that a response of the waterflea monitor is also likely to be close to lethal levels.

5.3.5. Field surveillance with waterfleas

So far, most of the effort on the waterflea monitor was dedicated to reduction of the above-mentioned practical bottlenecks and optimization of the experimental conditions. Figure 38. reflects a typical response of the waterflea monitor during a 1 week exposure at the monitoring station Lobith.

Figure 38. A typical response and its 99% confidence limit for a 4 h trend of the waterflea monitor during field application at the monitoring station of Lobith.



Preliminary analysis of one data set revealed a 24 h cycle. The variation that is left after application of moving averages for 24- and 4 h periods is less than 10% and follows a normal distribution. In Figure 38., a dynamic alert level was calculated retrospectively as a 99% confidence level.

Until recently, responses were often disturbed by false alerts. Data sets obtained in the near future will be used to improve

calibration of the dynamic alert level. To decrease the number of false signals, the level must be reached in both chambers before a response is classified as an alert.

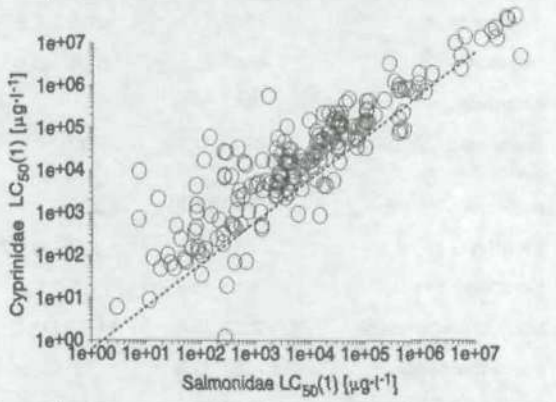
5.4. Discussion and conclusions

In the more polluted seventies, a rheotaxis fish monitor did not induce alerts over 3½ years of testing (Koeman et al. 1978) but the current fish early warning produced several alerts in recent years. However, experiences with fish early warning indicate that the monitor is likely to respond to concentrations that are close to lethal values, generally above the 1 to 10 $\mu g \cdot l^{-1}$ level. These thresholds will not be exceeded frequently at the average and peak concentrations currently measured in the Dutch Rhine (Hendriks 1993). This has invoked two directions for application of dynamic assays. The first aims to register episodes close to their emergence. Biological early warning may be useful at municipal and industrial effluents, especially upstream of calamity basins. In a pilot study, application of the fish monitor to several industrial effluents is being tested. So far, the dilution required for fish to survive the average water quality of the effluent in terms of suspended solids, microbes, salts and other microcontaminants hampers detection of episodes (Hof, personal communication).

The other direction is implementation of biological early warning systems that are more sensitive, at least to groups of chemicals not covered by the current fish monitor. The carp family, used in our project, is sometimes thought to be less sensitive in comparison to other major fish families, in particularly Salmonidae (e.g. Tucker and Leitzke 1979). Figure 39. indicates that acute lethal concentrations of both families are rather similar, so that differences, if present, must lay in the sublethal range.

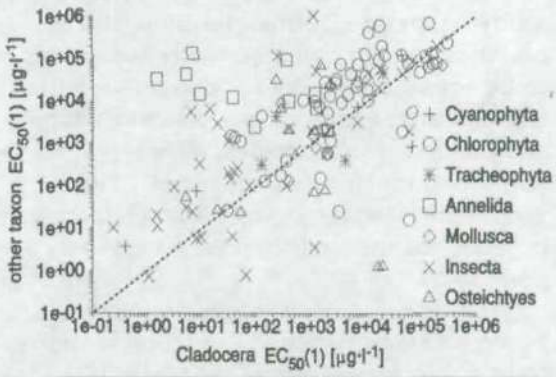
This is supported by tests with a fish monitor at Bimmen that is located on the other bank of the river near Lobith. This system registers ventilation of Salmonidae and yielded several alarms in 1991 (Stein, personal communication). This may be attributed to a higher sublethal sensitivity of

Figure 39. Lethal LC50(1) concentrations for Cyprinidae and Salmonidae (Pilli et al. 1989). Data represent geometric means for several species, lifestages and experimental conditions, normalized to 1 d exposure.



Salmonidae as well as to incomplete mixing of upstream influents.

Figure 40. Sublethal EC50(1) concentrations for Cladocera and other taxa (Pilli et al. 1989, B.K.H. 1990), geometric means for several species, lifestages and experimental conditions, normalized to 1 d exposure.



Slooff et al. (1983) and Slooff and Canton (1983) carried out assays with several taxa under similar conditions and demonstrated that sensitivity to several compounds varies widely but that Daphnia tends to be among the most susceptible laboratory genus. This is confirmed by Figure 40., which indicates that static EC50(1) values for Cladocera tend to be among the lowest. Analogous to past developments in static assays, the current

set needs to be extended with available bacteria (see e.g. Van Hoof et al. 1991) or algae monitors, to cover at least a minimum of compounds and species. Currently, various systems are being tested at different locations along the German Rhine (Schmitz 1992).

Extending the monitoring program to smaller organisms also will help to shorten response times, partly due to the higher surface:volume ratios. Yet, systems with smaller species sometimes require conditions, such as temperature or suspended solids loads, that are different from those prevailing in the field. Furthermore, some of these systems respond on a subindividual level, and the significance of these so-called biosensors for the aquatic community that one (cl)aims to protect may be insufficient. And so systems that measure concentrations instead of effects may result. This may be useful if compounds cannot be identified by traditional methods or if the monitor merely serves as a trigger for more sophisticated chemical analysis only. Development of very sensitive monitors should, therefore, not be overemphasized.

Instead, existing whole-organism monitors may be improved to increase their reliability under harsh field conditions. Meanwhile, an alert of the current fish and waterflea monitors should be taken very seriously as it is likely to be close to lethal levels.

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