RIVM report 607501 001/2001

A revised procedure to concentrate organic micro-pollutants in water

J. Struijs and R.E. van de Kamp

This investigation has been performed by order and for the account of the Directorate-General of the RIVM, within the framework of project 607501, Risk Assessment Ecosystems

Abstract

A new procedure to concentrate chemical pollutants in surface water samples is tested against 27 chemicals of varying physico-chemical and biological properties. A comparison is made to former procedures that have developed since 1994. The method has been applied since 1996 in measuring the toxicity of surface water samples in the framework of the project Geographic Representation of Ecotoxicological Effects of Substances. The test substances include hydrophobic chemicals with a (polar) narcotic mode of action, pesticides, surfactants and organotin compounds. The efficiency improved from 30 % to 60 % in terms of chemical recovery.

Preface

This report finalizes the development of extraction and concentration methods for testing the unknown cocktail of organic micropollutants from high volume surface water samples. Extraction methods are quite common in the investigation of environmental pollutants. The components are accumulated onto a solid (substrate) or into medium which is usually an organic solvent. Generally, this is no obstacle to perform a chemical or physical measurement. In many analytical techniques concentrating the components is essential for detection, and often it is desirable to separate the organic micropollutants from their aqueous environment.
To be implemented in the framework of the project Geographic Representation of Ecotoxicological Effects of Substances, extracts of organic micropollutants in *water* are required. The reason is that biological measurements in these extracts are carried out. As a consequence the extracts should be compatible to bioassays and at the same time contain the organic micropollutants in concentrations up to three orders of magnitude higher than in the original water sample.
During optimising the method to prepare the so-called “water concentrates” over a period of several years, we received support from our colleagues of the analytical laboratories LAC and LOC of RIVM. In arbitrary order we thank Arnold van de Beek, Rob Zwartjes, Elly Dijkman, Elbert Hogendoorn, Rob Ritsema and Luuk Fokkert. We also thank Pim Leonards and Willem van Loon for their stimulating discussions and recommendations at the Department of Analytical Chemistry of the Free University of Amsterdam.

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Samenvatting

Een concentreringsprocedure voor oppervlaktewatermonsters, die routinematig wordt toegepast bij monitoring van milieutoxiciteit, werd verbeterd en gevalideerd. Sinds 1996 vindt deze monitoring plaats en vanaf de zomer van 2000 worden volgens deze verbeterde procedure concentraten bereid van de onbekende cocktail aan organische micro-verontreinigingen in een oppervlaktewatermonster. De concentratiestap is nodig om de toxiciteit met behulp van een set van geminiaturiseerde bio-assays te kunnen meten.

De herziene concentreringsprocedure werd vergeleken met de vorige door het testen van de efficiëntie waarmee 27 chemicaliën kunnen worden geconcentreerd. De set van testverbindingen vertoont een grote variatie in fysisch-chemische en biologische eigenschappen en bevat hydrofobe stoffen met een (polair) narcotisch werkingsmechanisme, pesticiden, surfactanten en organotin verbindingen. De belangrijkste bevindingen zijn:

De opbrengst van het narcotische testmengsel, met daarin o.a. vluchtige en sterk adsorberende verbindingen, laat een opvallende verbetering zien, nl. van 18 % naar 60 %.
De opbrengst van pesticiden is ca. 70 %, evenals in de vorige procedure. Voor het eerst werden surfactanten en organotinverbindingen in het testprogramma opgenomen. De opbrengst van het anionogene LAS en het non-ionogene octaethyleenglycol monotetradecyl ether, die model staan voor de meest gebruikte wasmiddelen, bedraagt respectievelijk 40 % en 80 %.
De opbrengst van organotinverbindingen blijkt nihil, waarmee bevestigd wordt dat de extractieprocedure niet geschikt is voor metalen.
Het resultaat van de chemische opwerking is geschikt voor het stelsel van toxiciteitsmetingen dat de feitelijke meting van het milieumonster vormt: de opgewerkte watermonsters blijken voldoende compatibel met de bio-assays.
De praktische uitvoerbaarheid van de opwerkingsmethodiek blijkt aanzienlijk verbetererd ten opzichte van de oude methode (minder tijdrovend, verminderd gebruik van dure materialen) waardoor de aangepaste procedure beter geschikt is voor monitoring van toxisch risico in oppervlaktewater.

Summary

A concentration procedure for surface water samples, being applied on a routine basis in monitoring environmental toxicity, was improved and validated. This monitoring started in 1996 and since July 2000 this revised procedure is being applied to concentrate the unknown cocktail of organic micropollutants in surface water. This pretreatment is necessary for measuring the toxicity by means of a set of micro-bioassays.

The new concentration procedure has been compared against the former through testing the concentration efficiency for 27 chemicals. These test substances vary widely in physico-chemical and biological properties and include hydrophobic chemicals with a (polar) narcotic mode of action, pesticides, surfactants. The most important results are:

The recovery for the narcotic cocktail, including a.o. volatile and strongly adsorbing compounds, improved remarkebly: from 18 % to 60 %. The recovery of pesticides remained at the same level as the former procedure, i.e. 70 %. For the first time, surfactants and organotin compounds were included in the test programme. The recovery of the anionic LAS and the nonionic octaethylene glycol monotetradecyl ether, representing the majority of the surfactants used in the industrialized world was 40 % and 80 %, respectively.
The recovery of organotin compounds was zero, which confirms that the method is not suitable for metals.
The result of the chemical part of the whole procedure suits the toxicity measurements which constitute the actual measurement of the environmental sample: it was demonstrated that the concentrated water samples are compatible to bioassays.
Regarding ease of conductance, the method has improved considerably with respect to the former procedure (less time consuming, lower use rate of expensive materials) and is therefore more suitable for monitoring toxic risk in surface water.

1. Introduction

Since several years, research under the name of pT (toxic potency) is ongoing to measure toxic pressure in the aquatic ecosystem. The aim of pT is to quantify toxic risk in samples of surface-water in terms of the Potentially Affected Fraction (PAF) of species, exposed above the no-effect level. Acute toxic effects are measured directly (see Figure 1) by means of miniaturised aquatic toxicity tests (so-called toxkits), without identifying organic micropollutants (Roghair et al., 1997). The method has also been named measured Potentially Affected Fraction (PAF) to indicate that bioassays are employed for measuring toxic effects at varying concentration factors with different toxicity tests of a variety of test organisms. From a set of concentration factors, the PAF is calculated for the original water sample.

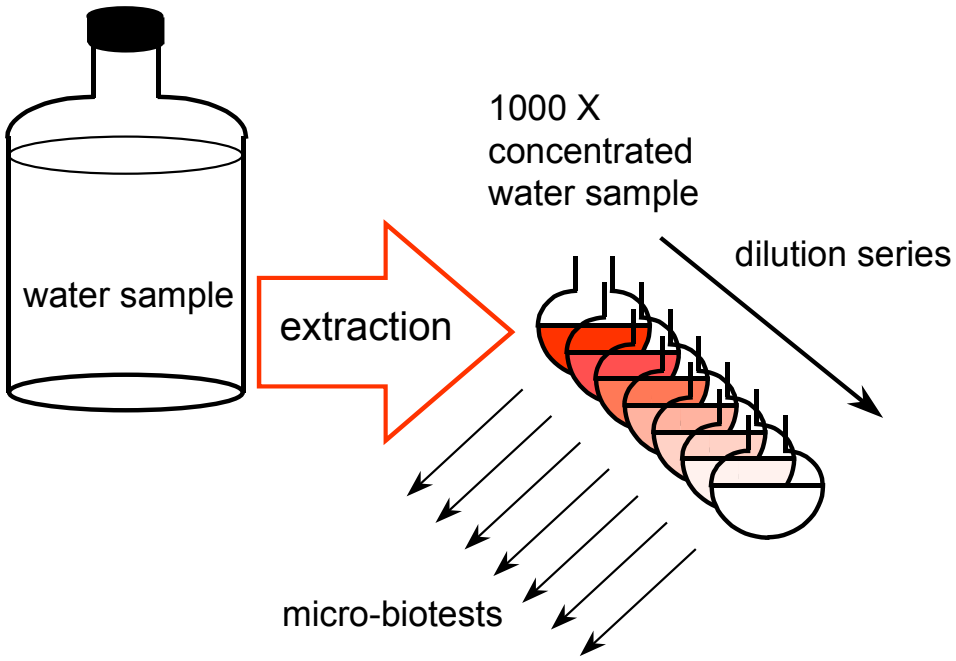

Figure 1 Procedure to obtain acute toxicity data related to an unknown cocktail of organic micropollutants in a surface water sample (Roghair et al., 1997).

Attempts to improve the procedure have focused both on improving the micro-bioassays and on the preparation of a thousand-fold aqueous concentrate of the environmental cocktail. It is essential that the medium for the concentrated chemicals be water - and not a solvent - in view of compatibility to micro-bioassays. The preparation of a concentrated water sample consists of four stages:

1. solid phase extraction of the organic toxicants with a mixture of the resins XAD-4 and XAD-8;
2. elution with one bed volume acetone;
3. removal of the bulk of acetone by means of a Kuderna-Danish (K-D) distillation;
4. transfer of the residue to a small volume of water; subsequent purging for 20 minutes to bring down the acetone concentration in the water sample below a non-toxic level.

For many organic chemicals this approach has yielded satisfactory results, but the weakness of the method is the loss of (semi-)volatile and/or hydrophobic substances. Loss due to volatilisation is caused by 20 minutes of purging with nitrogen, which is necessary to prepare a concentrated water sample for bioassays. Through purging, residual acetone is removed to such an extent that its contribution to toxic effects is negligible, also in a blank concentrated water sample (mineral water that has gone through the procedure).
Attempts to reduce volatilisation losses by applying extraction with super-critical carbon dioxide as an alternative for acetone were successful if the hydrophobic organic chemicals are concerned. It was shown with test mixtures of pesticides, however, that this approach is not suitable for more polar or ionised substances (Struijs et al., 1998). Many pesticides belong to that category as well as chemicals with an amphiphilic nature, such as detergents. As these substances may significantly contribute to toxic pressure, we decided to discard the super-critical carbon dioxide modification of the XAD solid phase extraction[1]. Provided a fair recovery of more polar substances is retained, we decided to accept some loss of volatile hydrophobic chemicals. This was chosen in preference to a good recovery of hydrophobic chemicals in combination with a poor yield of polar organic chemicals.
Here we report on optimising the solid phase extraction procedure with XAD and acetone, as a strategy to cover most relevant organic micropollutants in surface water, including pesticides and surfactants.

[1] Extraction with super-critical carbon dioxide has been successfully applied in sophisticated analytical methods to analyse several pesticides in soil matrices. Unfortunately, for each chemical a peculiar mode of operation is required in the procedure, which differs considerably per chemical. There is no single common method that covers most pesticides.

2. Revising the extraction/concentration procedure

In Table 1 the different stages of the XAD/acetone approach to concentrate organic micropollutants are summarised. The intended procedure for sorption of organic pollutants onto XAD and subsequent elution with acetone no longer requires a purging step at the end of the procedure. The left column represents the method as has been employed for several years in the framework of the project Geographic Representation of Ecotoxicological Effects of Substances (Roghair et al., 1997). In the right column modifications are listed to improve the method. A series of range finding experiments with varying water/XAD ratios are conducted to find the optimal procedure to remove acetone. After distillation, the small residue contains a cocktail of organic micro-pollutants - once present in a 60 L water sample - which is dissolved in a water/acetone mixture. The volume of the residue is small (usually less than 0.3 mL) when compared to the amount of water to which it is finally transferred to make up the concentrated water sample of 60 g. If the volume of the residue after distillation is sufficiently small, dissolving it in 60 mL of water will lead to a low concentration of acetone in the water sample. Below a specified level, it may cause negligible effects in the micro-biotests. On the condition that this level is not reached, the decision could be made to cancel the purging step in the procedure.

Table 1. Summary of the procedure to prepare a concentrated water sample and proposed modifications

Method for a 60 L sample (Struijs et al., 1998)	Modifications
Solid phase extraction 120 mL XAD-4/8; Contact time: 24 hr	Solid phase extraction Amount of XAD-4/8 reduced by a factor in the range between 2 – 10; Contact time prolonged (up to 48 hr)
Separation of the XAD resins from the water sample, subsequent drying of the XAD in a petridish under a gentile air stream overnight.	No changes.
Elution with one bed volume of acetone.	Elution with 1.7 bed volume of acetone. Scaling the size of the elution column according to the volume of XAD, keeping the contact time equal.
Storage of eluate in separated portions	Storage of eluate as one portion
Kuderna Danish distillation of a certain portion of eluate shortly before an intended micro-bioassay;	Single Kuderna Danish distillation of eluate with a smaller equipment, shortly before *all* micro-bioassays;
Uptake of the distillation residues in 60 mL water and subsequent purging with nitrogen during 20 min.	Uptake of the distillation residue in 60 mL water (total). *No purging with nitrogen.*

The aim is an optimised extraction method based upon XAD/acetone that is still manageable in monitoring activities. This includes a higher concentration efficiency than could be achieved before (Struijs et al., 1998), easier performance and a lower chance of false positive results. The following should be checked:

- The acetone concentration in the 1000-fold concentrated water sample is below a specified level, so ensuring that water samples, concentrated to a level of at least 500 times, are compatible to micro-bioassays.
- The efficiency of the solid phase extraction is not reduced by naturally occurring substances in the surface water, such as humic acids.

3. Materials and methods

3.1 Range finding experiments

The rate of adsorption onto the XAD resins was investigated with a test mixture of hydrophobic chemicals, varying in volatility and hydrophobicity. Mineral water was spiked with concentrations listed in Table 2. To optimise the method, varying amounts of XAD resins per volume surface water were applied and tested with respect of mixture N chemicals. During the sorption process, water samples were taken at intervals for chemical analysis. The analytical methods are given in Appendix 2.

3.2 The revised method tested in the recovery experiments

Appendix 3 contains a detailed description of the revised extraction/concentration procedure (SOP ECO/303/02 and SOP ECO/310/01). Briefly, the procedure to produce an acetone concentrate of organic micropollutants from a large volume of surface water (SOP ECO/303/02) consists of the following:

A 60 L surface water sample, without filtering, is mixed with 7.5 mL XAD-4 and 7.5 mL XAD-8 and distributed over 10 L borosilicate vessels;

On a rotary equipment the vessels are rolled for at least 48 hr;

The XAD particles are sieved and dried overnight under a gentile air stream. The loss of water during the drying process is measured by weighting the XAD;

The dried XAD is packed in an elution column;

Elution with 1.7 bed volume acetone (25 mL) is carried out to obtain acetone samples, which can be either stored or immediately processed according to SOP ECO/310/01.

SOP ECO/310/01 describes the procedure to treat the acetone eluate with the intention to convert it to a concentrated water sample, compatible to micro bio-assays:

Kuderna Danish distillation to remove acetone;

Uptake of the distillation residue in a small volume of mineral water to achieve a 1000-fold water concentrate;

Measuring the acetone concentration in the concentrated water sample to verify that a maximum level is not exceeded.

In Appendix 4 the differences between the former (SOP ECO/303/01 and SOP ECO/310/0) and the new procedure (SOP ECO/303/02 and SOP ECO/310/01) are summarised in a table.

3.3 Recovery of test mixtures

Details on the XAD resins and the physico-chemical properties and quality of the test chemicals were summarised earlier (Struijs et al., 1998). Water samples (10 L) were prepared from commercially available mineral water (Spa Blauw). They were spiked with mixture N (chemicals with a narcotic mode of action) according to Table 2, pesticides mixture A

(analysed with gas-chromatography, Table 3) and pesticides mixture B (analysed by means of HPLC, Table 4). The composition of the chemical mixture was determined, after elution in the acetone phase and in the water phase at the end of the procedure. Six replicates of mixture N were tested to determine the reproducibility of the procedure. Single analytical measurements of pesticides in the original water were done to check the efficiency of sorption onto the XAD resins after 48 hr. This was not done for mixture N because depletion characteristics were already known from the range finding experiments.
The procedure was tested in duplicate with 10 mg/L humic acid added to mineral water, which served as a surrogate surface water sample. The procedure was repeated again (duplicate) with real surface water, sampled from the Amsterdam-Rhine Canal. In the experiments with surrogate surface water and real surface water, depletion after the solid phase extraction of the chemicals was not measured.

3.3.1 Chemical analysis

Water samples containing mixture N chemicals were extracted with hexane and measured with gas chromatography (GC). The test chemicals in the acetone phase were measured directly. Water and acetone samples of mixture A were diluted in acetone and directly analysed with GC. Water and acetone samples of mixture B were diluted (acetone samples at least 10 x) in water and directly analysed with High Performance Liquid Chromatografic (HPLC).
The concentration of acetone in the concentrated water samples was analysed with GC. More details are given in Appendix 2.

3.4 Recovery of single chemicals

Recovery experiments with only one chemical added to water were performed with three surfactants and two organotin compounds (Table 5) in duplicate.
The three surfactants were only analysed in acetone and in the concentrated water samples. The concentrations of the spiked surfactants were too low for the applied analytical procedure to obtain the sorption efficiency directly from depletion data.
Recovery experiments with the organotin compounds were performed only in mineral water. Because of low yields, the experiments with humic mineral water and the real surface water sample were cancelled.

3.4.1 Chemical analysis

A “single surfactant” in the sense of one molecular structure is usually not available, but only as a mixture of homologues and isomers. Therefore a semi-specific analysis was applied.

3.5 Auxiliary analysis and measurements

Acetone in concentrated water samples was analysed with GC-FID (see Appendix 2). During the pT project as well over some period of the project Geographic Representation of Ecotoxicological Effects of Substances, we have collected a set of data on the residue volume

after KD-distillation and on the acetone content in the final thousand-fold concentrated water samples.
Loss of test chemicals from loaded XAD resins, acetone and concentrated water samples was monitored. Loaded XAD resins were stored in petri dishes at 4 C in an excicator, eluates were stored in glass bottles at – 20 C and the concentrated water samples (without headspace) were stored in flasks at 4 C.

Table 2 Mixture N, (polar) narcotic substances

Narcotic mixture	added concentration (µg L^{-1})
1,4-dichlorobenzene	10
Hexachloroethane	1
1,3,5-trichlorobenzene	2
3,4-dichlorotoluene	10
1,2,3-trichlorobenzene	4
3-chloronitrobenzene	16
2,4-dichloroaniline	45
1,2,3,4-tetrachlorobenzene	2
3,4-dichloro-nitrobenzene	15
2,4,6-trichloroaniline	16
Pentachlorobenzene	0.3

Table 3. Mixture A, pesticides (analysis: GC)

Pesticides A	Added concentration (µg L^{-1})
Mevinphos	100
Lindane	10
Diazinon	10
m-parathion	10
Fenchlorphos	1
Chlorfenvinphos	10

Table 4. Mixture B, pesticides (analysis: HPLC)

Pesticides B	Added concentration (µg L^{-1})
Metoxuron	10
Diuron	10
Azinphosmethyl	10
Linuron	10
Triazophos	1

Table 5. Chemicals tested individually

Chemical	Added concentration (µg L^{-1})
Sodium 1-dodecanesulfonate	20
Sodium dodecanebezenesulfonate (LAS)	25
Octaethylene glycol monotetradecyl ether	40
Triphenyltin	2
Tributyltin	2

4. Results

4.1 Range finding experiments

The difference in depletion rate between the old and new procedure is given by Figure 2. Results for other XAD/water ratios are given in Appendix 4. The most hydrophobic chemicals, 1,2,3,4-tetrachlorobenzene and pentachlorobenzene have the highest and the chloronitrobenzenes the lowest rates of disappearance from the aqueous phase. Nevertheless, the decay curves for all these organic substances, varying in hydrophobicity and volatility over more than 2 orders of magnitude, are sufficiently close to each other to lump the results and to compare directly the different XAD/water combinations.

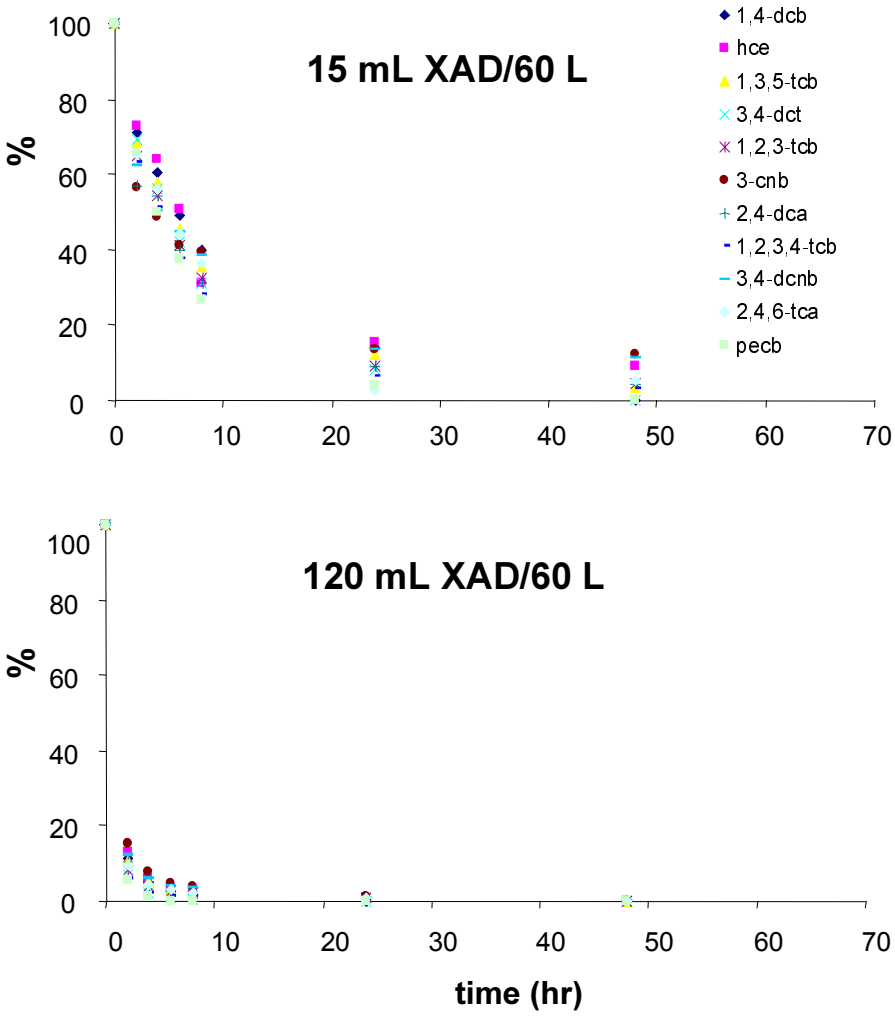

Figure 2 Depletion of 11 hydrophobic chemicals (mixture N) from a 60 L water sample as they adsorb onto XAD resins in the new (15 mL XAD) and the former (120 mL XAD) procedure.

Per mixture one depletion curve was calculated by taking the average of the different compounds of mixture N. The first order rate constant of disappearance from the water phase due to adsorption onto XAD is consistently proportional to the amount of XAD (Figure 3).

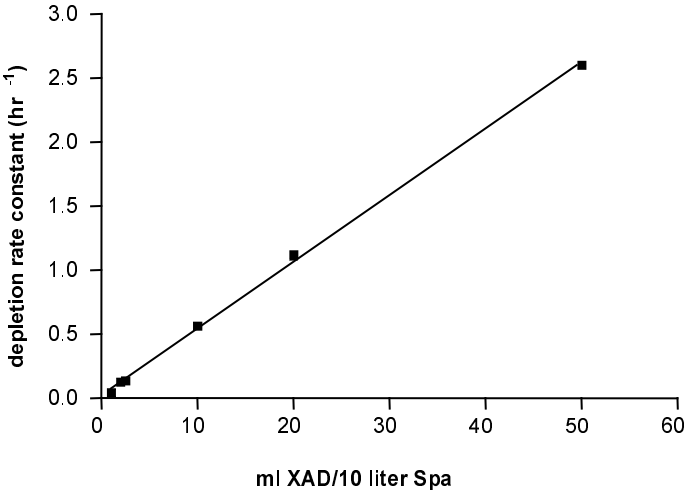

Figure 3 The sorption rate constant is proportional to the amount of XAD per volume water sample. From these results it can be derived that after 48 hr extraction with 1.2 or 1.9 mL XAD/10 L water, 95 %, respectively, 99 % depletion is achieved.

Considering these results the decission was made to extract the complex cocktail of organic micropollutants from a 60 L water sample with only 15 mL XAD in stead of 120 mL. From the depletion plots it was also concluded that the reduction of adsorptive capacity should be compensated by prolonging the contact time from 24 to 48 hr.

4.2 Recovery experiments

4.2.1 Mixture N in mineral water

Results from range finding experiments, also conducted with mineral water, were confirmed by 88 % recovery, which is the average of all replicates of eleven chemicals. The standard deviation per chemical is always below 8 % (Table 6a).
The distillation step causes major losses for 1,4-dichlorobenzene, hexachloroethane and 1,3,5-trichlorobenzene: two-third or more of these compounds was lost. Also half of 3,4-dichlorotoluene disappeared during distillation. Losses exceeding 50 % are accompanied by a relatively low reproducibility (~10 %), which reflects the impact of the distillation step on volatile chemicals. However, the majority of the chemicals return in the concentrated water samples to a large extent. The final recovery averaged over the eleven chemicals and all replicates is 58 %.

Table 6a. Recovery of (polar) narcotic substances (mixture N) from mineral water

Chemical	% in aceton eluate		% in concentrated water	
	Recovery	s.d. (n = 6)	Recovery	s.d.(n = 6)
1,4-dichlorobenzene	88	6	27	9
Hexachloroethane	79	3	18	10
1,3,5-trichlorobenzene	84	4	33	11
3,4-dichlorotoluene	84	6	48	7
1,2,3-trichlorobenzene	89	6	60	6
3-chloronitrobenzene	83	2	79	6
2,4-dichloroaniline	98	7	83	5
1,2,3,4-tetrachlorobenzene	93	4	69	6
3,4-dichloro-nitrobenzene	81	3	74	7
2,4,6-trichloroaniline	87	5	77	6
Pentachlorobenzene	92	6	69	9

Table 6b. Recovery in concentrated water sample for surrogate and real surface water samples.

Chemical	Mineral water + 10 mg/L humic	Amsterdam-Rhine Canal
	Average (duplicates)	Average (duplicates)
1,4-dichlorobenzene	32 (26/38)	28 (39/17)
Hexachloroethane	21 (16/26)	21 (24/18)
1,3,5-trichlorobenzene	36 (28/44)	41 (44/38)
3,4-dichlorotoluene	47 (38/57)	52 (57/48)
1,2,3-trichlorobenzene	53 (44/63)	63 (73/53)
3-chloronitrobenzene	73 (69/77)	80 (82/79)
2,4-dichloroaniline	81 (82/81)	90 (92/88)
1,2,3,4-tetrachlorobenzene	60 (51/68)	67 (71/62)
3,4-dichloro-nitrobenzene	71 (69/73)	80 (80/81)
2,4,6-trichloroaniline	69 (62/76)	81 (84/78)
Pentachlorobenzene	66 (61/72)	64 (65/63)

4.2.2 Mixture N in surrogate and real surface water

The recovery in acetone eluate (data not shown) averaged over the duplicates of eleven chemicals in mineral water containing 10 mg/L humic substances is 90 %, which is slightly higher than in pure mineral water (88 %). Apparently, the humic substances do not reduce sorption of this type of chemicals onto XAD. In the real world sample, the average recovery is 92 % (data not shown). Table 6b lists the final recovery for all mixture N chemicals.
The final average recovery of all chemicals in duplicate from humic mineral water is 55 % and from the Amsterdam-Rhine Canal sample 61 %. Both values are around the results obtained with pure mineral water (58 %).

4.2.3 Pesticides mixture A

After 48 hr of contact with XAD, one combined water sample was taken from the six experiments in order to estimate to which extent the spiked pesticides were withdrawn from the mineral water. The high withdrawal percentages (Table 7a) are in agreement with results obtained in the former procedure (Struijs et al., 1998), except mevinphos, which remained for 37 % in the water phase, being consistent with 60 % recovery in acetone. With the former procedure, using an eight-fold higher amount of XAD in 24 hr, 6 % was not extracted (Struijs

et al., 1998), but surprisingly only 7 % was found in the acetone. However, those results had been obtained from a single experiment from which no conclusions could be drawn as analytical-chemical artefacts could have influenced the the results with mevinphos.

Table 7a. Solid phase extraction of mixture A pesticides in mineral water. Recovery in acetone eluate and in the concentrated water sample (average and standard deviation).

Mixture A in mineral water	% not adsorbed	% in acetone eluate (n = 6)	s.d. % in acetone eluate	% in water sample (n = 5)	s.d. % in conc. wat. sample
Mevinphos	37	60	6	50	3
Lindane	7	95	7	64	6
Diazinon	6	88	4	63	4
m-parathion	3	96	8	77	3
Fenchorphos	0	95	5	58	6
Chlorfenvinphos	5	91	7	81	3

Table 7b. Solid phase extraction of mixture A pesticides in humic mineral water and in a real world water sample. Recovery in acetone eluate and in the concentrated water sample.

Mixture A in (surrogate) surface water	Mineral water + 10 mg/L humic material		Amsterdam-Rhine Canal	
	% in acetone eluate (n = 2)	% in conc. water sample (n = 2)	% in acetone eluate (n = 2)	% in conc. Water sample (n = 2)
Mevinphos	28 (27/28)	24 (24/24)	26 (24/29)	24 (23/25)
Lindane	100 (98/101)	64 (74/55)	105 (100/111)	76 (85/67)
Diazinon	94 (91/97)	61 (65/58)	86 (80/93)	68 (72/64)
m-parathion	100(96/105)	83 (89/76)	109 (109/110)	89 (93/85)
Fenchorphos	101 (96/105)	63 (70/57)	104 (95/112)	79 (86/71)
Chlorfenvinphos	98 (94/102)	78 (85/72)	98 (88/108)	81 (82/79)

The sorption efficiency for mevinphos decreases considerably if (competing?) substances other than this test chemical are present. This is apparent both in mineral water with humic material and in Amsterdam-Rhine Canal water (Table 7b) where the recovery in acetone eluate is only 27 %. These reduced yields indicate that in pure mineral water, the sorption capacity of the applied amount of XAD is already critical for mevinphos. With the other pesticides again high yields were obtained.
The duplicates in Table 7b did not differ more than 10 % from each other. The average of all replicates was almost equal to the results in mineral water and higher than 80 %.The average of final recovery (concentrated water sample) was 65 % for mineral water, 62 % with humic mineral water and 69 % for Amsterdam-Rhine Canal water.

4.2.4 Pesticides mixture B

From three pairs of flasks, combined water samples were taken to determine the depletion percentage. Metoxuron was not completely removed by XAD: one quarter (Table 8a) remained in the mineral water, which was confirmed by the recoveries found in the acetone. In the former procedure with eight times more XAD per water volume, complete withdrawal from the aqueous phase was observed and a fairly high recovery in acetone (85 %) after elution with 4 bed volumes (Struijs et al., 1998). The relatively small amount of XAD in the revised method may be critical for this pesticide. In experiments with surrogate and real world water samples (Table 8b) the yields were further reduced. Probably as a result of

competition between trace levels of metoxuron and other (humic) substances in relatively high concentrations. These results have a similarity to mevinphos in mixture A.

Table 8a. Mixture B pesticides extracted from mineral water: recoveries in acetone and water.

Mixture B in mineral water	% not adsorbed onto XAD (n = 3)	% in acetone eluate (n = 6)	% in conc. Water sample (n = 6)
Metoxuron	26 (s.d. = 7)	63 (s.d. = 6)	59 (s.d. = 5)
Diuron	7 (s.d. = 3)	81 (s.d. = 4)	74 (s.d. = 2)
Azinphosmethyl	5 (s.d. = 2)	91 (s.d. = 3)	79 (s.d. = 6)
Linuron	7 (s.d. = 3)	88 (s.d. = 4)	80 (s.d. = 4)
Triazophos	5 (s.d. = 5)	102 (s.d. = 3)	81 (s.d. = 7)

Table 8b. Mixture B pesticides extracted from humic mineral water and real surface water. Duplicate recoveries in acetone eluate and in the concentrated water sample.

Mixture B in (surrogate) surface water	Mineral water + 10 mg/L humic material		Amsterdam-Rhine Canal	
	% in acetone eluate (n = 2)	% in conc. water sample (n = 2)	% in acetone eluate (n = 2)	% in conc. Water sample (n = 2)
Metoxuron	46 (44/47)	37 (33/40)	51 (47/56)	50 (46/54)
Diuron	74 (73/74)	56 (51/62)	77 (75/79)	68 (69/68)
Azinphosmethyl	100 (101/99)	70 (63/76)	91 (89/93)	69 (83/55)
Linuron	91 (91/91)	66 (59/73)	88 (86/89)	72 (82/63)
Triazophos	107 (107/107)	74 (65/82)	102 (102/103)	74 (95/53)

The average recovery in acetone of mixture B is 85 % for mineral water, 84 % for humic mineral water and 82 % for Amsterdam-Rhine Canal. In the concentrated water samples, the recoveries are respectively 75 %, 60 % and 67 %.

4.2.5 Surfactants and organo-tin compounds in single experiments

From surface-active compounds we may expect that sorption onto XAD resins is complete. However, the semi-specific analytical method applied here did not allow determining the degree of depletion. Only in the concentrated samples of acetone and water the capability of the procedure was evaluated. Negligible (sodium 1-dodecanesulfonate) and only partial (LAS) recovery in the acetone phase (Table 9a) was measured. Failure to appear in the acetone concentrate should be attributed to lack of affinity of the anionic surfactants to acetone. There is a strong indication that sodium 1-dodecanesulfonate could not be released from the XAD as it was insoluble in acetone, while LAS seemed only sparingly soluble. Sorption of the non-ionic surfactant and release from the XAD through acetone elution must have been complete, as full recovery (Table 9a/b) was observed in the concentrated water sample.

Because sodium 1-dodecanesulfonate and the organotin compounds (Table 9a) gave very low results in mineral water, experiments for surrogate and real surface water were cancelled. From mineral water, whether or not fortified with humic substances, slightly less than 50 % of LAS was analysed in acetone. Further processing into a concentrated water sample yields approximately 40 %. From Amsterdam-Rhine Canal water, however, less than 30 % was found. Lower yield may be explained from other substances present in a real water sample, interfering either with the solid phase extraction or with the analytical procedure.

Table 9a. Recovery of individually tested chemicals in mineral water

Test chemical	% not adsorbed onto XAD (n = 2)	% in acetone eluate (n = 2)	% in conc. Water sample (n = 2)
Sodium 1-dodecanesulfonate	n.d.	6 (6/5)	3 (3/2)
Sodium dodecanebezenesulfonate (LAS)	n.d.	45 (36/53)	38 (33/44)
Octaethylene glycol monotetradecyl ether	n.d.	n..d.	104 (103/105)
Triphenyltin	13 (13/14)	< 2	< 2
Tributyltin	13 (12/13)	< 2	< 2

Table 9b. Recovery of surfactants (%) from surrogate and real surface water

Test chemical	mineral water + 10 mg/L humic material		Amsterdam-Rhine Canal	
	Acetone eluate (%)	Conc. water sample (%)	Acetone Eluate (%)	Conc. Water sample (%)
Sodium dodecanebezenesulfonate (LAS)	53 (49/58)	47 (44/50)	29 (36/21)	24 (31/17)
Octaethylene glycol monotetradecyl ether	n.d.	86 (89/83)	n.d.	75 (78/72)

4.3 Compatibility with bio-assays

A relatively small volume of acetone is typical for the revised method: only 25 mL of acetone ideally contains the organic compounds once present in a 60 L surface water sample. The advantage is that the K-D distillation equipment can be considerably smaller than the installation applied in the former procedure. Moreover, the distillation vessel includes a calibrated tube enabling to observe the volume of the residue, which remains after boiling has ceased within the temperature window of 65 - 70 C. Often this volume is less than 0.2 mL (Figure 4).

This residue consists mainly of water and acetone in approximately equal amounts, apparently being an azeotropic mixture with a boiling point significantly higher than 70 C. Assuming that the residue contains 50 % acetone, 0.2 mL residue dissolved in 60 mL water would result in a concentrated water sample with 0.17 volume % acetone. The measured concentration of acetone in 32 water concentrates is distributed according to Figure 5.

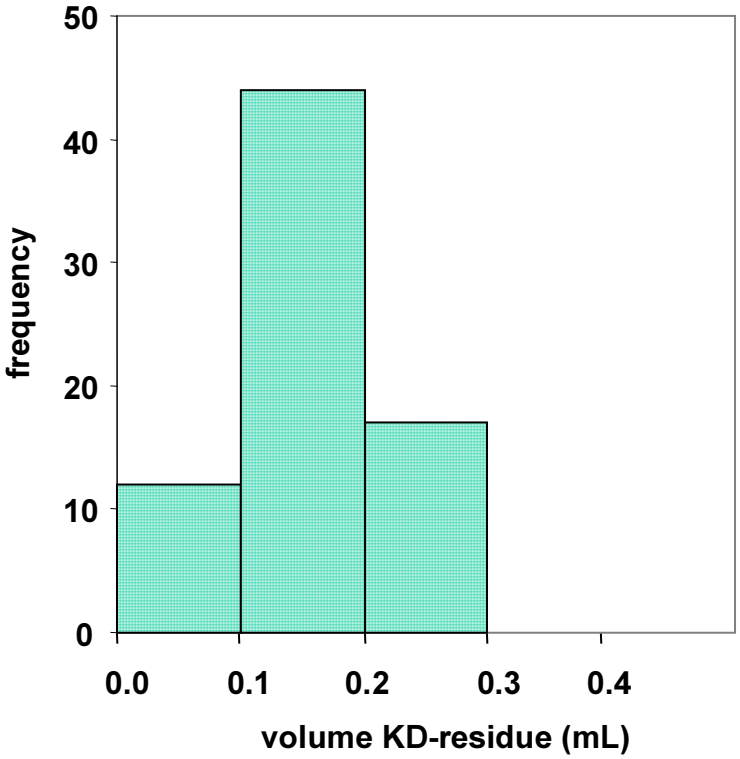

Figure 4 Distribution of the residue volume (n = 73) after distillation of 25 mL acetone eluate. Average is 0.20 mL, standard deviation 0.06 mL.

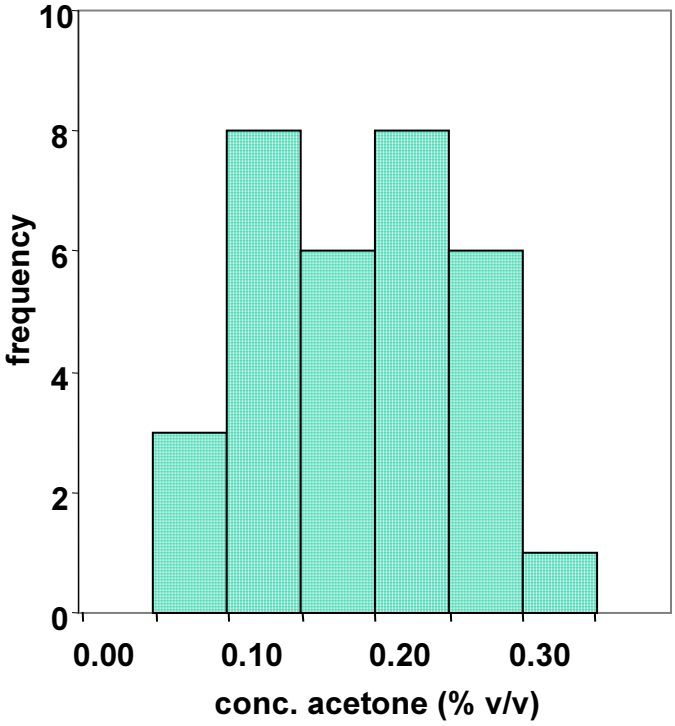

Figure 5 Histogram of 32 acetone concentrations in concentrated water samples of 60 mL. Average is 0.19 %, standard deviation = 0.07; 95 percentile = 0.29 %.

4.4 Storage of XAD, acetone or concentrated water samples

Before June 99, in the project Geographic Representation of Ecotoxicological Effects of Substances, a 60 L surface water sample was converted into 120 mL acetone which was subdivided in portions of 20 mL. Each portion was treated according the old procedure, which includes KD-distillation and purging to obtain a 10 mL concentrated water sample appropriate for a scheduled bioassay. This allowed different bio-assays to be conducted at different occasions on a time scale of several months or even longer. Since January 2000 the new procedure is applied on a regular basis in the project. Until bio-assays are carried out by RIVM and RIZA, undivided concentrated samples are stored as acetone eluates of ca 30 mL at -20 C. The new procedure, however, requires biological testing within a shorter period because the whole acetone concentrate is concentrated to yield one batch of water concentrate. In practice, if different laboratories are involved, some time will have elapsed before all bioassays are carried out. Therefore it is necessary to know how long a concentrated water sample can be stored at 4 C.

Simulated water concentrates were stored in glass vessels at 4 C over a period of 100 days, during which the presence of mixture N chemicals was monitored (Figure 6). The most hydrophobic chemicals (penta-, tetra- and one of the trichlorochlorobenzenes) have lost 20 to 30 % of the initial concentration after one week, most likely due to sorption onto the glass wall (Figure 6). A storage time longer than two weeks for narcotic substances is not recommended, as very hydrophobic chemicals might have disappeared for more than 50 %.

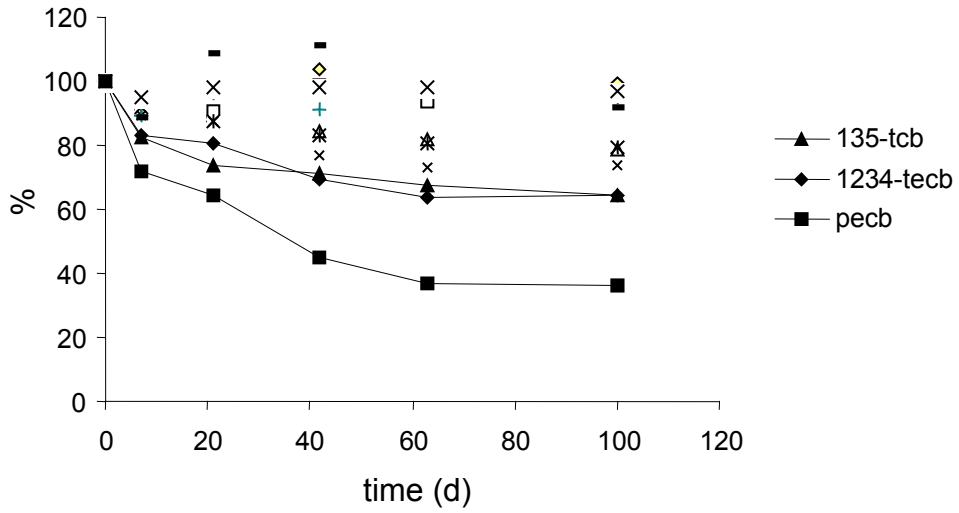

Figure 6. Concentration of mixture N chemicals in water kept in glass vessels stored at 4 °C for several months

An alternative for acetone as a medium for storage is XAD. We tested the capacity of XAD 4/8 to retain organic chemicals over a longer period. The amount of a mixture of some volatile compounds of mixture N and some pesticides on XAD 4/8 was determined

periodically (Figure 7). The results suggest that storing the concentrates on XAD in petri dishes is a good alternative for storing acetone eluates.

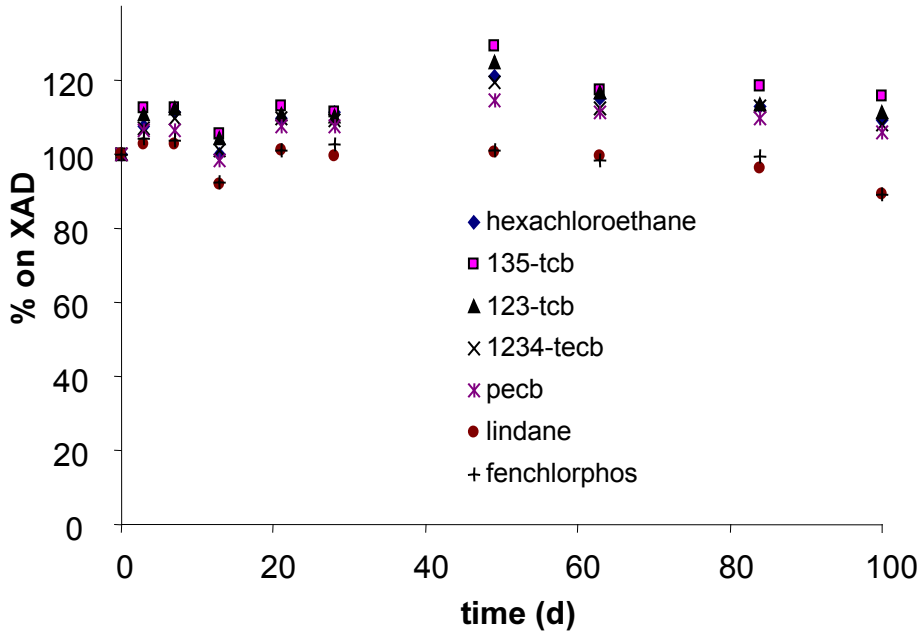

Figure 7. Relative amounts of hexachloroethane, 1,3,5- and 1,2,3- trichlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, lindane and fenchlorphos on XAD resins stored in petri Relative dishes kept in an excicator at 4 ℃

5. Discussion and conclusions

The test chemicals of mixture N, A and B vary over more than 5 orders of magnitude in octanol-water partition coefficient, 9 in Henry's law constant, almost 4 in the water solubility and 6 in vapour pressure. The results are compared to data reported by who applied the former procedure to the same chemicals.
The improvement with the hydrophic chemicals of mixture N is most profound: while in the old method no more than 18 % was collected in the water concentrate, 60 % is found in this study. The average recovery of the 11 pesticides is 70 %, which does not seem to be an improvement when compared to 71 % found previously. However, the last figure is probably an overestimation as for four pesticides values far exceeding 100 % had been obtained. Although set to 100 % for calculating the average, this will bias the average recovery.
The new method, when only pesticides are concerned, is at least as satisfactory as the former. Possibly, the new procedure is slightly less suitable for more polar pesticides, but this compensated by better results for the more hydrophobic non-polar pesticides.
If all chemicals of mixtures N, A and B are considered, we find 43 % in the former and 64 % in the new method.

It is unknown how the surfactants behave in the former method. LAS and octaethylene glycol monotetradecylether are the best representatives for all anionic and nonionic surfactants, respectively, which comprise 80 % (approximately in equal amounts) of the total surfactant volume. With respect to LAS (~ 40 % recovery in this study) and octaethylene glycol monotetradecyl ether (~ 90 % recovery), the volume of sodium 1-dodecanesulfonate (zero recovery) can be neglected.

It is very likely that the organotin compounds can not be concentrated in acetone in the old method. In the new version with low amounts of XAD, we have found complete sorption. Failure to release these compounds from the XAD through acetone elution is certainly the reason for zero recovery. It seems very likely that in the former procedures equally low results would have been obtained. The XAD/acetone procedure seems not suitable for metals and probably neither for their organic derivates (see also Struijs et al., 2000).

Conclusion 1: Regarding recovery, the new method is an improvement: 64 % versus 43 % in the former procedure. The average of all organic compounds investigated in this study (N, A, B and the surfactants) is 62 %; including the two organotin compounds 58 %.

Conclusion 2: Regarding ease of application, the new method is less laborious and material consuming than the former method and therefore more suitable for monitoring toxic stress in surface water.

Conclusion 3: The chemical yield is approximately 60 %. It is principally unknown how much toxic potency is lost in the procedure. The unknown cocktail in surface water varies per sample and we do not know which toxic chemicals will (partly) not show up in an aqueous concentrate. From the group of 25 organic chemicals tested in this study insight is gained in the methods potency to extract and concentrate toxic chemicals from surface water.

5.1 Sorption efficiency influenced by humic substances

The recovery of (polar) narcotic chemicals does not seem to have been negatively affected by humic substances. Two out of eleven pesticides, mevinphos and metoxuron, are partially sorbed onto XAD. In the old procedure they were completely withdrawn. Results with surrogate and real surface water samples indicate that modifications in the solid phase extraction (lower amount of XAD, longer time for sorption) have some negative influence. In the new procedure, however, recoveries in acetone eluate, i.e. 60 % for mevinphos and 63 % for metoxuron, were more consistent and even better than in the old procedure: 7 % and 85 % respectively (Struijs et al., 1998). Note that these two pesticides distinguish from other test compounds: stand above others by one order of magnitude in water solubility, while log K_{ow} is the lowest of all test chemicals of mixtures N, A and B.

Conclusion 4: The more hydrophilic the chemicals are, the lower the recovery is in the solid phase extraction, in particular if naturally occurring substances are present, like humic acids. Older versions of the procedure are probably more suitable for this special category of chemicals.

5.2 Development over the last five years

The degree of complexity of the procedure is entirely determined by maintaining the compatibility to bioassays after having concentrated organic micropollutants. Sixteen chemicals have been investigated over five years in four different procedures.
Table 10 summarises the progress made by reducing - and finally by eliminating - the purging step in the procedure.

Table 10. Recoveries of 16 chemicals by 4 different procedures

Procedure	ECO/076/00 (1993)[1]	ECO/303/00, ../01 ECO/310/00 (1997/1998)[2]	XAD & SFE (1997/1998)[3]	ECO/303/02 ECO/310/01 (1999)[4]
Sorption	120 ml XAD	120 ml XAD	30 ml XAD	15 ml XAD
Elution	120 ml acetone	120 ml acetone	Super-critical CO_2	30 ml acetone
Distillation	No	K-D	No	Micro K-D
Test chemical | Purging	6 hr	20 min	No	No
1,4-dichlorobenzene	0	0	5	29
Hexachloroethane	0	0	5	21
1,3,5-trichlorobenzene	0	0	24	36
3,4-dichlorotoluene	0	0	26	49
1,2,3-trichlorobenzene	0	0	37	61
3-chloronitrobenzene	0	49	58	79
2,4-dichloroaniline	4	49	49	83
1,2,3,4-tetrachlorobenzene	0	0	48	69
3,4-dichloro-nitrobenzene	0	48	60	75
2,4,6-trichloroaniline	0	44	53	76
Pentachlorobenzene	0	6	43	69
Lindane	0	36	12	64
m-parathion	79	55	21	77
Fenchlorphos	0	20	27	58
Chlorfenvinphos	95	52	79	81
Diuron	91	66	6	74
Average	**17**	**26**	**34**	**63**

[1] results reported by Collombon et al. (1997).
[2] results (polar) narcotic chemicals by Collombon et al. (1997); pesticide data are the average of results reported by Collombon et al. (1997) and Struijs et al. (1998).
[3] data (polar) narcotic chemicals is the average of results reported by Collombon et al. (1997) and Struijs et al. (1998); pesticide data are reported by Struijs et al. (1998).
[4] This report

5.3 Chemicals not investigated in this study

5.3.1 Industrial chemicals

Hydrophobic chemicals are concentrated to a high extent provided that the volatility is not too high. From the results obtained with mixture N it is clear that if Henry's law constant exceeds 100 Pa m^3/mol or if the vapour pressure is higher than 30 Pa, the recovery falls below 50 %.

In view of the results obtained with pentachlorobenzene, the method is expected to be suitable for other hydrophobic chemicals such as phthalates, PCB's, PAH's and chlorinated dioxins and dibenzofuranes.

For very polar or (partly) ionized organic chemicals, considerations as given for the pesticides and surfactants may generally apply.

5.3.2 Pesticides

Bentazone and diclobenil were not included in recovery assessments. Although bentazone is entirely ionized at neutral pH, still 30 % could be accumulated onto XAD in former procedures (Collombon et al., 1997 and Struijs et al., 1998). However only with SOP ECO/076 it was possible to release bentazone from the XAD, probably because in that procedure the "acetone eluate" appeared to contain tens of percent of water, resulting in a

more polar solvent. In a later version of the XAD/acetone approach – and it is almost certain this is also true for the latest version – the yield in the aqueous concentrate is zero. For other highly polar or ionized pesticides, for example pentachlorophenol, SOP ECO/076/00 might give a higher recovery efficiency than with the new approach. Diclobenil was found at levels above 100 % in any concentrate, probably due to analytical errors (Collombon et al., 1997). In view of its physico-chemical properties, a high recovery would be expected also in the new procedure.
Higher recoveries are obtained the more the test chemical is hydrophobic. The methods seems particularly suitable for compounds like lindane or pentachlorobenzene and conceivably also for hexachlorobenzene, DDT and the drins.

5.3.3 Surfactants

In terms of production volume and emission pattern, LAS is the most important anionic surfactant. The difference between LAS and dodecylsulphonate is the benzene ring of LAS, which increases the hydrophobicity of the molecule. Both substances seem to have a low affinity for acetone, but the presence of a benzene ring in the LAS molecule probably enhances somewhat the affinity for acetone. This may be the explanation for partly release from XAD by acetone, where dodecylsulphonate completely failed to desorp. It is therefore very likely that other alkylsulphonates can not be eluted with acetone either. However, the category of alkylsulphonates is negligible as water pollutant compared to LAS.
Octaethylene glycol monotetradecyl ether, tested in this study, is also a good representative for all nonionic surfactants produced in the EU, with a high share of all surfactants produced. Because of their physico-chemical properties, we may expect that with this procedure all other nonionic surfactants are also concentrated in acetone and water to a high extent.
Cationic surfactants, applied as fabric softeners in households, were not involved in this study. Although their share of emission to water is only 10 % of all surfactants, their contribution to toxic stress is considered important.

5.3.4 Are concentrated water samples compatible with bioassays?

In the former procedure, purging was considered a precautionary measure, if not a requirement to sufficiently eliminate acetone. Although a specific analytical method to measure the concentration of acetone in water concentrates was not operational before, it is now explainable why purging was required[2].

We have implemented an analytical method to measure the acetone concentration in concentrated water samples. Sufficient data on the acetone concentration in the concentrated

[2] The residue of a K-D distillation consists of approximately equal amounts of water and acetone in which the cocktail of organic micropollutants is dissolved. The azeotropic character of this mixture implies that boiling at 65 -75 C ceases. In the former procedure this residue is considerably more volumous than the 0.2 mL we find in the new procedure. However it has to be transferred to an equal volume of water in order to make up the "concentrated water sample" which is the starting liquid for bio-assays. If a relatively high volume of residue,

water samples could be collected to verify that omitting the purging step in the new procedure is allowed. Measured levels are compared to acetone toxicity reported by Vaal and Folkerts (1998).

The 95 percentile of the acetone concentration in the thousand-fold concentrated water sample is 0.29 volume percent and the mean value is 0.19 %. The no-effect concentration derived by Vaal & Folkerts is however 0.15 volume percent. This means that possibly some bio-assays, if conducted with the highest concentration factor, may be affected. In practice, concentration factors higher than 500 are not encountered when determining an effect criterion in bio-assays. Even for the blank, being mineral water that has to be highly concentrated to observe toxic effects, a concentration factor of 500 is rare. To derive some intended effect criterion, concentrated water samples are considered valid up to 500 times as there is more than 95 % change that the acetone content is below the no-effect concentration.

Conclusion 5: The new method for preparing concentrated water samples is compatible to the biological part of the pT methodology.

containing tens of procents acetone, is dissolved in water, the actone concentration will exceed the no-effect level. Purging is then necessary to further reduce the acetone concentration.

References

OECD (1971). Pollution by Detergents: Determination of the Biodegradability of Anionic Synthetic Surface Active Agents. OECD, Paris, 1971.

Posthuma, L., Suter, G.II. and Traas, T.P. (eds.) (2001). Species Sensititvity Distributions in Ecotoxicology, CRC Press Pensecola (due 20 Dec. 2001).

Roghair, C.J.. Struijs J. and De Zwart D. (1997). Measurement of toxic potency in fresh waters in the Netherlands; Part A: Methods. RIVM-Report nr. 607504 004. Nat. Inst. Publ. Health & Environment. The Netherlands.

SOP ECO/076/00 (1993). Voorschrift voor het gebruik van XAD-hars bij de extractie van organische verontreinigingen uit oppervlaktewater.

SOP ECO/303/00 (1996) Voorschrift voor het concentreren van organische microverontreinigingen uit water met behulp van XAD-harsen.

SOP ECO/310/00 (1997) Opwerking van een acetonconcentraat tot een watermonster voor aquatische toxiciteitstoetsen.

Struijs. J., Van de Kamp. R. and Hogendoorn. E.A. (1998). Isolating organic micropollutants from water samples by means of XAD resins and supercritical fluid extraction. RIVM-Report nr. 607602 001. Nat. Inst. Publ. Health & Environment. The Netherlands.

Struijs. J., Ritsema R., Van de Kamp., R. and De Zwart, D. (2000). Toxic pressure in surface water. A pilot of new monitoring techniques. RIVM-Report nr. 607200 003. Nat. Inst. Publ. Health & Environment. The Netherlands.

Van Beusekom, S.A.M., Admiraal, W., Sterkenburg, A. and De Zwart, D. (1999), ECO notitie 98/09 HANDLEIDING PAM-TEST. In Dutch.

US-EPA. 1984. AQUIRE: Aquatic Information Retrieval Toxicity Database. Project Description, Guidelines and Procedures by R.C.Russo and A.Pilli, EPA 600/8-84-021, U.S. Environmental Protection Agency,.Environmental Research Laboratory-Duluth, Office of Research and Development, Duluth, MN.

Wickbold, R. (1973). Analytical determination of small amounts of nonionic surfactants. Tenside Detergents 10, 179-182.

Appendix 1 Mailing list

1	Directie RIVM, Prof. ir. N.D. van Egmond
2	Ir. M.R.de Potter (KEMA, Arnhem)
3	Dr. G. Schoeters (VITO, België)
4	Dr. A.J.Murk (LUW, Wageningen)
5	Dr. W.M.G.M. van Loon (RIKZ, Haren)
6	Dr. P.E.G. Leonards (RIVO, IJmuiden)
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16	Dr. W. Slooff (RIVM/CSR)
17	Hoofd Bureau Milieu- en Natuurverkenning, Drs. R.J.M. Maas
18	Hoofd Laboratorium voor Water en Drinkwater, Ir. A.H.M. Bresser
19	Dr. L. van Liere (RIVM/LWD)
20	Hoofd Laboratorium voor Ecotoxicologie, Drs. J.H. Canton
21	Dr.Ir. D. van de Meent (RIVM/ECO)
22	Dr. A. Sterkenburg (RIVM/ECO)
23	Dr. Ir. W. Peijnenburg (RIVM/ECO)
24	Drs. D. de Zwart (RIVM/ECO)
25	Dr. A. B. Breure (RIVM/ECO)
26 -27	Auteurs
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37 - 48	Reserve-exemplaren t.b.v. het Laboratorium voor Ecotoxicologie

Appendix 2 Information on (test) chemicals

Table A1. Trade mark and purity of the surfactants

Compound	mol.formula	CAS nr	trade mark	purity
Sodium 1-dodecanesulfonate	$C_{12}H_{25}NaO_3S$	2386-53-0	Fluka	>99%
Sodium dodecanebezenesulfonate (LAS)	$C_{18}H_{29}NaO_3S$	25155-30-0	Fluka	80%
Octaethylene glycol monotetradecyl ether	$C_{30}H_{62}O_9$	27847-86-5	Fluka	>99%

Table A2. Trade mark and purity of organotin compounds

C ompound	Mol.formula	CAS nr	trade mark	purity
Tri-n-butyltin acetate	$C_{14}H_{30}O_2Sn$	56-36-0	Strem-chemicals	98%
Trifenyltin acetate	$C_{20}H_{18}O_2Sn$	900-95-8	Strem-chemicals	97%

Table A3. Trade mark and purity of humic acid

Compound	Mol.formula	CAS nr	trade mark	purity
Humic acid sodium salt	-	1415-93-6	Janssen chimica	tech.

Analytical Methods

Narcotic mixture

Water samples were extracted with n-hexane with an internal standard. The extractinon time was 5 minutes. Depending from the expected concentration dilutions were made in n-hexane with internal standard. Acetone samples were, when necessary, diluted in acetone and directly analysed. Quantitative Gas Chromatografic analyses were performed with a Carlo Erba Strumentazione, series HRGC 5300 GC, with a splitter SL 516, an autosampler AS800 and an Alltech Econocap SE54 analytical column (30 m, 0.32 mm ID, 0.25 µm film). Detection was performed by a Carlo Erba Strumentazione Electron Capture Detector. Peaks were integrated by the Millipore Maxima 820 (v3.30) integration system. Concentrations were calculated with a calculation program Calwar.xls.

GC conditions were: sample size 1.0 µl, splitless injection, split time and bottom flow depends from expected concentration and detector sensibility, injection port temperature 250 ºC, column temperature programmed from 45 ºC to 70 ºC at a rate of 20 ºC/min, 1 min at 70 ºC, from 70 ºC at a rate of 5 ºC/min to 170 ºC and at a rate of 20 ºC/min to 275 ºC. Detector temperature: 290 ºC. Helium gas flow rate 2.0 mL/min.

Pesticide mixture A

Water and acetone samples were diluted in acetone and directly analysed. Quantitative Gas Chromatografic analyses were performed with a Carlo Erba Strumentazione, series HRGC 5300 GC, with a splitter SL 516, an autosampler AS800 and an Alltech Econocap SE54 analytical column (30 m, 0.32 mm ID, 0.25 µm film).

Detection was performed by a Carlo Erba Strumentazione Electron Capture Detector. Peaks were integrated by the Millipore Maxima 820 (v3.30) integration system. Concentrations were calculated with a calculation program Calwar.xls.

GC conditions were: sample size 1.0 µl, splitless injection, split time and bottom flow depends from expected concentration and detector sensibility, injection port temperature 250 ºC, column temperature programmed from 1 min at 150 ºC, from 150 ºC at a rate of 30 ºC/min to 200 ºC, at a rate of 5 ºC/min to 240 ºC and at a rate of 20 ºC/min to 275 ºC. Detector temperature: 290 ºC. Helium gas flow rate 2.0 mL/min.

Pesticide mixture B

Water and acetone samples were diluted (acetone samples at least 10 x) in water and directly analysed. Quantitative High Performance Liquid Chromatografic analyses were performed with a LDC Analytical CM4000 HPLC-pump, a Marathon autosampler and a Kratos Spectroflow 757 UV-detector.
A Chrompack Chromspher 5 PAH (20 cm, 5 µm particle size) column was used. Peaks were integrated by the Millipore Maxima 820 (v3.30) integration system. Concentrations were calculated with a calculation program Calwar.xls.

HPLC conditions were: sample size 10 µl, elution with 60% acetonitril - 40% water (isocratic), flow rate 0.7 ml/min, detection wavelenght 210 nm.

Surfactants and organotin compounds

For anionic surfactants, such as sodium dodecylsulfonate and LAS, the analytical parameter responds to all homologues and isomers as so-called Methylene Blue Active Substances (MBAS). In this method a salt with methylene blue is formed which is soluble in chloroform and measured with a spectrophotometer (OECD, 1971). For the nonionic surfactant, octaethylene glycol monotetradecyl ether, the semi-specific analytical response is Bismuth Active Substances (BiAS), according to the tetra-iodobismuthate method of Wickbold (1973). This procedure consists of precipitation of the nonionic agents by bismuth containing reagent (Dragendorff reagent) and potentimetric titration of the bismuth content of the precipitate.
The concentration of surfactants was only analysed in the concentrated samples: acetone and the final water sample.
The organotin compounds were analysed in the original water samples after 48 hr, in the acetone eluates and in the concentrated water samples by means of gas chromatography – inductively couple plasma mass spectrometry (GC-ICPMS).

Acetone

Water samples with traces of acetone were directly analysed. Quantitative Gas Chromatografic analyses were performed with a Carlo Erba Strumentazione, series HRGC Mega 2 8560 GC, with a splitter SL 516, an autosampler AS800 and a Chrompack CPWAX57CB analytical column (25 m, 0.25 mm ID, 0.20 µm film).
Detection was performed by a Carlo Erba Strumentazione Flame Ionisation Detector. Peaks were integrated and concentrations were calculated by the Millipore Maxima 820 (v3.30) integration system.
GC conditions were: sample size 0.1 µl, split injection, bottom flow 125 ml/min, injection port temperature 240 ºC, column temperature 60 ºC (isotherm). Detector temperature: 250 ºC. Helium gas flow rate 2.0 mL/min.

Appendix 3 Standard Operating Procedures

SOP/ECO/303/02

STANDARD OPERATING PROCEDURE 303 FOR EXTRACTING ORGANIC MICROPOLLUTANTS FROM WATER SAMPLES WITH XAD RESINS

1 INTRODUCTION

Organic contaminants can be extracted from water samples using a 1:1 mixture of XAD-4 and XAD-8 resins (solid phase extraction). Desorption of the organic chemicals is achieved by elution with a small amount of acetone. The extract can be used for chemical analyses or, after further processing (SOP/ECO/310/01), for measuring toxic effects.

2 CHEMICALS

2.1 Methanol, p.a., Merck 1.06009
2.2 Acetone, p.a., Merck 1.00014
2.3 Spa blauw water
2.4 XAD-4, cleaned up [2], (KIWA-Nieuwegein)
2.5 XAD-8, cleaned up [2], (Supelite DAX-8, cleaned up by KIWA-Nieuwegein)

3 MATERIALS

3.1 Borosilicate flask (10 litre), with caps with teflon inlay
4.2 Funnels
4.3 Beakers
3.4 Elution column, glass, 300 mm x 10.5 mm ID with coarse frit, teflon stopcock and inlet joint (Supelco cat no. 64756).
3.5 Pasteur capillary pipet with a wide point
3.6 Vials with crimpcap (Chrompack)
3.7 Sieve, 50 μm, inert material
3.8 Freezer
3.9 Shaker
3.10 Petri dish Ø 10 cm
3.11 Crimper

4 PROCEDURE

4.1 Procedure to obtain an XAD4/8 mixture in the aqueous phase

Clean XAD 4 and 8 is stored in methanol. Before carrying out the solid phase extraction, the XAD must be transferred to the aqueous phase. Use for 60 litre of water sample 7.5 mL XAD-4 and 7.5 mL XAD-8. Pour the XAD slurries, using a funnel, into an elution column, starting with the XAD-4 so that XAD-8 is on top of XAD-4 in the column.

Wash the XAD with 2 bed volumes methanol (=30 ml) and 6 bed volumes Spa-blauw mineral water (=90 ml).

4.2 Solid Phase Extraction

Transfer the aqueous XAD4/8 mixture, using some water, to a clean beaker before distributing it over the various 10 litre flasks the 60 litre water sample is subdivided in. Add to each 10 litre sample flask 2.5 ml XAD4/8, using the pasteur pipet and a 10 ml measuring cylinder. Close the sample flasks.
Place the flasks on a shaker and shake, in the dark at 20°C, for at least 48 hours .
Sieve the XAD out of the water using the 50 μm sieve.

4.3 Drying the XAD

Dry the XAD as good as possible by wiping the bottom of the sieve with a tissue.
Transfer the XAD quantitatively to a clean petri dish (Ø 10 cm). Determine in advance the empty weight of the dish. Spread the XAD over the complete surface of the dish.
Place the petri dish during the night (18 hours) in a gentle air stream in a hood. Shake the petri dish gently a few times, during the drying time. The XAD is dry enough when it weights less than 4.5 gr.

4.4 Elution

Transfer the dried XAD to a clean elution column, length 300 mm, Ø 10.5 mm, using some acetone. Remove air bubbles by turning the column a few times. Elute the XAD slowly with ca 25 ml acetone. An elution time of at least 30 min. assures a high extraction efficiency.
Collect the eluate in a vial (30 ml), close it and store the vial in a freezer.

Literature

[1] RIVM Veiligheidsregels, BAM / 007
[2] Beveren, J. van: Voorschrift voor de XAD isolatie in watermonsters van 50 tot 300 liter, deel 2: de opwerking; KIWA Nieuwegein, juni 1989

SOP ECO/310/01

PROCEDURE FOR TREATMENT OF AN ACETONE CONCENTRATE, CONTAINING ORGANIC MICROPOLLUTANTS: CONVERSION INTO A WATER CONCENTRATE SUITABLE FOR FOR MEASURING TOXIC EFFECTS

1 INTRODUCTION

An acetone concentrate, containing organic micropollutants, obtained by a solid phase extraction from a water sample using XAD4/8 resins (SOP/303/02), can be processed to a water concentrate suitable for measuring toxic effects, using a Kuderna Dänish distillation.

2 CHEMICALS

2.1 Acetone, p.a (Merck 1.00014)
2.2 Spa blauw water
2.3 Boiling chips (Merck 1.07913)
2.4 Aluminum foil
2.5 Dutch Standard Water (DSW)

3 MATERIALS

3.1 Water bath (for example: a magnetic stirrer/heat plate, and a beaker filled with water + magnetic stirring bar)
3.2 Kuderna Dänish Sample Concentrator (Supelco, Receiving Vessel 2 ml cat nr. 6-4723, Flask 250 ml cat nr. 64729, Solvent Recovery Condenser cat nr. 64839).
3.3 Thermometer (100°C)

4 PROCEDURE

4.1 KD-distillation procedure

Switch on the stirrer/heat plate and heat the water temperature up to **65-70 ºC.** Turn on the cooling water of the condenser. Transfer the acetone eluate quantitatively to the 250 ml flask with receiving vessel, which is a calibrated tube, and add one boiling chip and **0.5 ml** Spa-blauw water. Place the condenser on top of the 250 ml flask and start the distillation by placing the KD apparatus in the water bath. Wrap up the flask with aluminum foil. Keep the temperature of the water bath during the whole distillation at **65-70 ºC. Stay alert.** Stop the distillation as soon as the residue stops boiling (ca 0.2 ml). Remove **immediately** the receiving vessel and close it.

Transfer the residue, using a pasteur pipet, quantitatively to a 60 ml sample vial and fill up with DSW to 60 ml. Close the vial and store it in a refrigerator until conducting the bio-assays.

Appendix 4 Range finding experiments: decay curves