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Procedures for extracting organic micro-pollutants from water samples to monitor toxicological stress

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SAMENVATTING

Methoden werden gevalideerd die deel uitmaken van toxiciteitsmonitoring van het aquatische milieucompartiment. Een methode is recent toegepast in een gezamenlijke proefmonitoring van het RIVM en RIZA voor het karteren van de potentiële toxiciteit van Nederlandse oppervlaktewateren als de Potentieel Aangetaste Fractie van soorten ($PAF_{gemeten}$).
Drie gedeeltelijk in Standard Operating Procedures vastgelegde procedures voor het verkrijgen van een "waterconcentraat" van een oppervlaktewatermonster worden met elkaar vergeleken. Zij zijn alle gebaseerd op het toepassen van macro-reticulaire harsen van het type XAD bij het extraheren van organische micro-verontreinigingen uit water, maar verschillen in de wijze waarop de toxicanten naar de waterfase worden teruggebracht. De geschiktheid van de methoden werd geëvalueerd door de chemische opbrengst van goed gedefinieerde testmengsels met elkaar te vergelijken.

Het toepassen van een XAD-4/8 mengsel is geschikt voor alle teststoffen met log $K_{ow} \geq 2$. Bentazon was de enige teststof met een lagere partitiecoëfficiënt (log K_{ow} = -0,46). De hoeveelheid bentazone geadsorbeerd aan de XAD-4/8 harsen was 28%, wat verrassend hoog is gezien de nagenoeg volledige dissociatie bij pH = 7 (pK_a = 3).
In twee methoden wordt aceton toegepast om de toxicanten van het XAD te elueren; de wijzen waarop het aceton wordt verwijderd zijn echter verschillend. In beide methoden werd de verwijdering van aceton op een zodanig niveau gebracht dat de resulterende waterconcentraten sterk verhoogde gehaltes aan toxicanten bevatten maar verwaarloosbare - althans toxicologisch aanvaardbare - gehaltes aan aceton. Wegens vervluchtiging is aan de toegepaste destillatie- en afblaastechnieken onvermijdelijk een aanzienlijk verlies van teststoffen verbonden. Dit speelt vooral een rol indien de vluchtigheid van de teststoffen in termen van de Henry constante (H) in het gebied ligt tussen 0,1 en 100 Pa m^3 mol^{-1} (afhankelijk van de wijze waarop aceton verwijderd werd). Vrijwel volledig verlies werd geconstateerd voor de testchemicaliën met hogere waarden voor H.

Een derde methode is gebaseerd op het toepassen van super-critical fluid extraction (SFE) voor het desorberen van de toxicanten van XAD. Het mijden van (vaak toxische) organische oplosmiddelen heeft het voordeel dat destillatie of afblazen niet nodig is waardoor de methode geschikt is voor chemicaliën met H tot 2000 Pa m^3 mol^{-1} . De vluchtigheidsgrens, in termen van dampspanning, waarboven geen stoffen meer geconcentreerd kunnen worden is 3 Pa voor de Kuderna Danish modificatie en 100 Pa voor de SFE modificatie. Ofschoon de resultaten met SFE een voorlopig karakter hebben, lijken ze veelbelovend omdat met deze concentreringstechniek een grotere diversiteit aan chemicaliën bereikt kan worden.

SUMMARY

Methods are validated that are elemental in toxicity monitoring of the aqueous compartment. One method has recently been implemented in a joint pilot monitoring of an RIVM/RIZA project to map the toxic potency of Dutch surface waters in terms of the Potentially Affected Fraction of species (PAF measured).
Three procedures, partly laid down in Standard Operation Procedures, to obtain a "water concentrate" of a surface water sample are compared. They all rely on the use of macro-reticular resins of the XAD type to extract organic micro-pollutants from water, but are different in how the toxicants are returned to the water phase. The performance of the methods was evaluated by comparing the chemical recovery of well-defined test mixtures.

The use of an XAD-4/8 mixture is appropriate for all test chemicals with log K_{ow} = 2 or higher. Bentazone was the only test chemical with a lower octanol-water partition coefficient (log K_{ow} = -0.46). The amount of bentazone sorbed onto the XAD-4/8 resins was 28 %, which is high taken the almost complete dissociation at pH = 7 (pK_a = 3) into consideration. In two methods acetone is applied to elute the toxicants from the XAD, but differ in the technique to remove the acetone. In both methods elimination of acetone was achieved to such an extent that the resulting water concentrates have increased concentrations of most toxicants but a negligible - or at least toxicologically acceptable - content of acetone. The employed distillation and/or purging techniques are, however, inevitably coupled with a significant loss of chemicals due to volatilisation. This is significant if the volatility of the test chemicals, in terms of Henry's law constant (H), is in the range of 0.1 to 100 Pa m^3 mol^{-1} (depending on the method used to remove acetone) and almost complete disappearance was measured for chemicals with higher values of H.

A third method is based upon the use of super-critical fluid extraction (SFE) to desorb the toxicants from the XAD resins. Avoiding the use of toxic organic solvents has the advantage that chemicals with H up to 2000 Pa m^3 mol^{-1} are covered because distillation and purging are not necessary. The upper limit of volatility, in terms of vapour pressure, above which no compounds could be concentrated increased from 3 Pa (Kuderna-Danish distillation) to 100 Pa (SFE technique). Although results are preliminary, they suggest that in concentrating chemicals a greater diversity can be accomplished.

1. INTRODUCTION

1.1 General

To assess environmental quality in almost all environmental compartments, measuring systems have been implemented for the evaluation of the concentration of a limited number of chemicals. Because of the wide variety of occurring contaminants and the low availability of ecotoxicity data, such an approach does not allow for a realistic assessment of effects that might occur. For evaluating the state of the environment, it would be more helpful to measure the actual toxic stress, spatial and temporal, related to environmental exposure from the unknown mixture of toxic compounds.
In the Laboratory of Ecotoxicology of the RIVM methods are being developed to monitor toxicological stress on eco-systems (De Zwart et al., 1996). This research has been denoted as "the pT method", indicating the potential toxicity of the complex mixture of substances present in environmental samples. The aim is a measuring procedure which relies on an integrated biological parameter of a field sample, producing an index for the toxicological potency of it. In this context "integrated" refers to both - in principal - *all* occurring contaminants and the *combined* toxic effects they might have on occurring organisms.

During the early 70s, when water pollution reached its maximum level, acute toxic effects could be detected in some untreated river water samples and dilution was necessary to obtain a certain effect criterion. Presently, surface water most often has a too low toxicity to cause acute effects to be quantified directly with currently available techniques. Therefore, it is a prerequisite to be able 1) to concentrate a water sample and 2) to evaluate the toxicity in rather small samples of such "water concentrates". Both items are addressed in the project "the pT method"; this report describes methodology to prepare water concentrates.

The methodology to prepare a concentrate of water samples is not yet satisfactory, but progress can be made in the near future (Van Egmond et al., 1996). The aim is a concentration procedure which delivers an *aqueous* sample being directly appropriate for aquatic toxicity testing. At the current stage of development, such extraction and isolation methods are only feasible for the unknown cocktail of organic chemicals in water. Results in this report are attempts to validate what should be considered interim methods. One method has been applied previously (De Zwart & Folkerts, 1990), another is a modification of it and has been implemented in a joint pilot monitoring of an RIVM/RIZA project in 1996/97 (Roghair et al., 1997).

1.2 The environmental chemistry part of the pT project

Much attention has been given to the identification of chemicals and to the combined toxic effects in real water samples (e.g. Hendriks, 1996), rather than to validation of the extraction/concentration procedures. One important feature of validation is that it includes at least determining the chemical recovery of well-defined test mixtures which serve as surrogate water samples. The aim of the environmental chemistry part of the pT project was to evaluate the chemical recovery for different methods as a function of water solubility, vapour pressure and octanol-water partition coefficient. This evaluation enables the extraction/concentration part of the whole method to be assessed, compared and improved. All methods reported here rely on solid phase extraction by means of macro-reticular resins (XAD). Surrogate water samples were submitted to the different methods. Test mixtures were prepared in a reference water, for which a commercially available mineral water is used. One mixture consists of 12 hydrophobic chemicals with a wide range of physico-chemical properties, the second mixture contains seven pesticides. Two different procedures that are based upon desorption of toxicants through acetone elution and subsequent removal of acetone, are compared. By evaluating the chemical fate at several stages along the concentration procedure, weaknesses of each method are identified and opportunities for improvement indicated. A third procedure with super-critical fluid extraction (SFE), using super-critical CO_2 as an alternative for organic solvents such as acetone, is described. The use of organic solvents has the major drawback of being toxic itself or causing changes in toxic effects of organic micropollutants; therefore it has to be removed before conducting aquatic toxicity tests. It is expected that with respect to distillation and purging techniques, the SFE approach enables a recovery over a wider range of chemical properties and so a greater diversity of recovered chemicals to be submitted to toxicological testing, than is possible in the joint pilot monitoring of the RIVM/RIZA project.

In Chapter 2 an explanation is given how exposure from an unknown cocktail of toxicants can be expressed on the basis of measured toxicity. Practical implications with respect to concentrating toxicity in water are discussed. The selection of XAD as an accumulator of environmental toxicity and the use of mineral water as a reference are motivated. Chapter 3 contains the general principles of the procedures in short descriptions and schemes. Properties of the test chemicals, auxiliary materials and analytical methods are described in Annexes A to E. Recoveries of the test chemicals at various stages in the different procedures, are presented in Chapter 4. Critical steps in each procedure are identified. In Chapter 5 an evaluation of the different concentration procedures is made by comparing plots of chemical recovery versus a physico-chemical property. Recommendations for future research are made.

2. CONCENTRATION METHODS AND THE MEASURED TOXICITY OF A WATER SAMPLE

2.1 Expressing the exposure from an unknown cocktail of toxicants

The ultimate goal is to develop a method for treating water samples in a way that allows the evaluation of toxic potency. As the mixture of toxicants in surface water has an unknown composition, units like mg/L to relate the exposure to toxic effects are meaningless. Therefore, the toxicity of a water sample is expressed as the number of times the original sample has to be concentrated (or diluted) to meet a certain effect criterion in the toxicity test. It is convenient to employ a notation which is close to symbols that are common in aquatic ecotoxicology. In stead of the aqueous concentration **C** (f.i. in mg/L like in EC_{50}) the *concentration factor* $\mathbf{C^f}$ (dimensionless) is used to express the exposure:

$\mathbf{EC^f_{50}}$ is the **f**actor by which a water sample has to be **c**oncentrated to find **50** % **e**ffect in a standardised toxicity test;

$\mathbf{NEC^f}$ is the *minimum* **f**actor by which a water sample has to be **c**oncentrated to exceed the **n**o **e**ffect criterion in a standardised toxicity test.

If a sample has to be diluted to meet a specified effect criterion, by convention the parameter is between zero and one. The measured toxicity's for various aquatic species at various concentration factors are evaluated in terms of the measured Potentially Affected Fraction of species, PAF(measured) (see Roghair et al., 1997).

2.2 Concentrating toxicity in water: practical implications

Surface water, groundwater or rain usually have such low concentrations of toxicants that it is necessary to concentrate the samples in order to be able to observe an acute toxic effect. In principle, withdrawing sufficient water molecules from a water sample would achieve this. However, this is energetically unfavourable and manipulations and the use of auxiliary material are unavoidable. As a consequence, toxicants may be lost or external toxicity may be added. Attempts to reduce such undesirable changes in toxicity of the sample will put high demands on the purity, stability and removability of all auxiliary materials.
Practical restrictions are also determined by the applied biological test systems and the desired information derived from them. The design and sensitivity of each biological test and the number of different methods, may require such a high volume of a field sample that the procedure would be unsuitable for monitoring. A high recovery of toxic chemicals in the concentration procedure is therefore favourable for monitoring as it reduces the need to sample volumes of water that are not practical.

2.3 Demands on the concentration procedure

- Low addition of toxicants to the sample during treatment. From auxiliary materials like adsorbentia, solvents, glassware etc. toxicity may be released during the treatment of a water sample. If this is a severe problem, a very pure reference water sample may give a relatively high response. When the released impurities exceed the toxicity of the environmental contaminants, a small difference between field and reference would be observed. In addition, a high "procedure toxicity" may cause loss of accuracy and so an undesirably low differentiation among field samples. It is therefore recommended to limit the number and amounts of auxiliary chemicals. If a solvent is used, besides its purity special attention has to be given to its - preferably high - removability from water and its - preferably low - toxicity in water.
- Low loss of toxicants from the sample during treatment. Loss of toxicants during the concentration procedure is not expected to be proportional to the total amount of toxicity present in a water sample. If such a linear relationship would exist, *relative* exposure data would be obtained. Interface transport processes, like (de)sorption and volatilisation, however, are dependent on the physico-chemical properties of the chemical. Depending on these properties, toxicants may behave differently during the extraction/concentration process. Water samples may widely vary both in composition of organic substances and the related distribution of the physicochemical parameters. As a consequence, different cocktails may cause differences in EC^{f}_{50} values, due to physico-chemical phenomena rather than to differences in toxicity of the samples.
- Appropriate for monitoring toxicological stress on ecosystems. If the method is too laborious or too expensive, it will fail as an instrument for systematic diagnosis of toxicological stress on ecosystems. Also this criterion will put special demands on the use of auxiliary agents, cleaning procedures and the use of glassware and instruments.

2.4 Solid phase extraction with XAD resins

The use of a mixture of two macro reticular resins, XAD-4 and XAD-8, for biomonitoring surface waters has been described by De Zwart & Polman (1993, 1994[a,b]). Briefly, their method to concentrate a surface water sample consists of solid phase extraction of the organic contaminants from the water sample, desorption through elution with approximately one bed volume of acetone and evaporating the acetone by lengthy purging with nitrogen. The volume of the residue was adjusted by adding a small amount of water to obtain a 1000-fold "water concentrate". This method (Figure 1) has been described in a standard operation procedure (SOP ECO/076, 1993).

A literature study on the use of XAD-4/8 (Struijs & Van Buren, 1995), confirmed the suitability of the XAD-4/8 mixture to concentrate a wide variety of organic micro-pollutants in the aquatic environment. Recovery experiments with hydrophobic test mixtures indicated that sorption onto this mixture of resins and desorption with acetone is satisfactory. In the procedure of De Zwart & Polman (1993), the final step, i.e. the removal of acetone which is required to obtain an aqueous sample that is appropriate for toxicological testing, is very problematic because many chemicals are lost due to volatilisation. Therefore, it was tentatively decided not to optimise the procedure by searching for alternatives of XAD-4 and XAD-8, but to optimise the removal of acetone (or other suitable solvents if any) and to search for alternatives for organic liquids to desorp the contaminants from the XAD.

3. MATERIALS AND METHODS

Procedures are described in general terms and figures, which schematically represent the principles. Details on methodology and test chemicals can be found in Annexes A to E, in standard operating procedures and other literature references.

3.1 Auxiliary substances

All materials used to manipulate the surrogate water samples were clean and inert, consisting of either glass or stainless steel, as indicated in SOP ECO/076, SOP ECO/303 and SOP ECO/310.
Detailed information on XAD-4 and XAD-8 resins, such as properties and the purification procedure are given in Annex A.
Surrogate water samples were prepared from Spa blauw mineral water, which is commercially available in synthetic bottles. In view of the intended measurement of toxicity in aqueous field samples, this water proved satisfactory as reference water (Struijs & Van Buren, 1995).

3.2 Test chemicals and chemical analysis

Information on the test chemicals is given in Annex B. Hydrophobicity of the narcotic mixture ranges from log k_{ow} is 2.6 to 4.8 and the volatility in terms of Henry's law constant[1] ranges from below 0.1 to more than 1000 Pa m^3 mol^{-1} and in terms of vapour pressure from 0.04 to 440 Pa. The composition of the narcotic test mixture is given by the left part of Table 1. This test mixture served both validation and the development of a new concentration methodology. The chemical analysis allows the simultaneous determination of all components in samples taken at different stages of the process. Pre-treatment of all compounds was similar and analysis was performed by one instrumental method. This cocktail allows a quick evaluation of experiments aimed at improvement of the procedure.
The composition of the pesticide mixture was chosen for validation only: to quantify the chemical recovery of seven well known pesticides. The composition of the pesticide mixture is shown in the first two columns of Table 2. Physico-chemical properties are given Annex B. In Tables B1 and B3 (Annex B) the quality of the test chemicals is given.
The preparation of stock solutions and the surrogate water samples is described in Annex C.
All water samples containing the narcotic chemicals were extracted with n-hexane p.a. and analysed by GC-ECD (De Groot et al., 1996). Two different instrumental methods were employed for the pesticide mixture. HPLC was applied to bentazone and diuron and GC for the other components. Details of the analytical procedures are given in Annex D.

[1] Henry's law constant is the air-water partition coefficient of a compound. It can be estimated from the ratio of the compound's vapour pressure (Pa) and its water solubility (mol m^{-3}). H/RT is the dimensionless air-water partition coefficient (c_g/c_w), with R is the universal gas constant (8.314 Joules K^{-1} mol^{-1}) and T is the absolute temperature (K).

3.3 The use of XAD resins and acetone to prepare an aqueous concentrate

Two methods, laid down in standard procedures, are visualised in Figures 1 and 2. Both methods have in common that a mixture of XAD4/8 resins and the solvent acetone are the media for concentrating organic chemicals before the intended water concentrate is prepared. The whole procedure consists of the following steps:

1) quantitative sorption of the organic contaminants onto XAD4/8 resins (solid phase extraction), which is similar in SOP ECO/076 and SOP ECO/303;
2) collection of the XAD resins (similar in SOP ECO/076 and SOP ECO/303) and drying (only SOP ECO/303);
3) elution of the organic chemicals with acetone according to either SOP ECO/076 or SOP ECO/303,
4) replacement of acetone with water, according to either SOP ECO/076 or SOP ECO/310.

3.3.1 Solid phase extraction and acetone elution (step 1-3)

The procedures rely upon solid phase extraction (SPE) using an 1:1 mixture of XAD-4 and XAD-8. In two respects there is a difference between SOP ECO/076 and SOP ECO/303 in obtaining an acetone eluate. The first procedure prescribes that after collecting XAD on a sieve, wet XAD be transferred directly into an elution column. According to SOP ECO/076, the first few mL is discarded which is water that can be visually discerned from the following acetone phase; the subsequently collected eluate (approximately 26 mL) contains 6 ± 2 mL water. According to SOP ECO/303, wet XAD is allowed to lose water overnight in an open petri dish under a gentle air stream in the hood. Evaporation of water causes a loss of weight by 6±2 g. Therefore, one bed volume (= 20 mL) is collected which contains less than 2 (?) mL of water.

3.3.2 Replacing acetone with water (step 4)

The main difference between the two procedures is how the chemicals in the acetone eluate are transferred to the water phase to obtain a "water concentrate". In SOP ECO/076 (Figure 1) a 6 hr period of purging with nitrogen is necessary to meet the criterion that the solvent concentration is below a specified maximum level[2]. After a Kuderna-Danish (KD) distillation according to SOP ECO/310 (Figure 2), only 20 minutes of purging is necessary to meet this criterion. The boiling point of acetone is at least 100 °C lower than the test chemicals (see Annes B).The simple KD technique enables a concentration of chemicals dissolved in a volatile solvent by a factor up to 100 to 200 (Van den Broek, 1990). After this concentration step only 20 minutes of purging is required to remove the residual acetone.

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[2] The upper limit of acetone concentration permitted in an aqueous sample was set to 1.2 g/L (1.5 mL/L), based on results from experiments with *Photobacterium phosphoreum, Brachionus calycifloris* and *Daphnia magna*. Lowest observed No Observed Effect concentration (NOEC) was measured with *D. magna*. This is a concentration 15 times higher than is permitted by the OECD guidelines (100 µL/L), but considerd acceptable in the development of a new technique. See Vaal & Folkerts (1998).

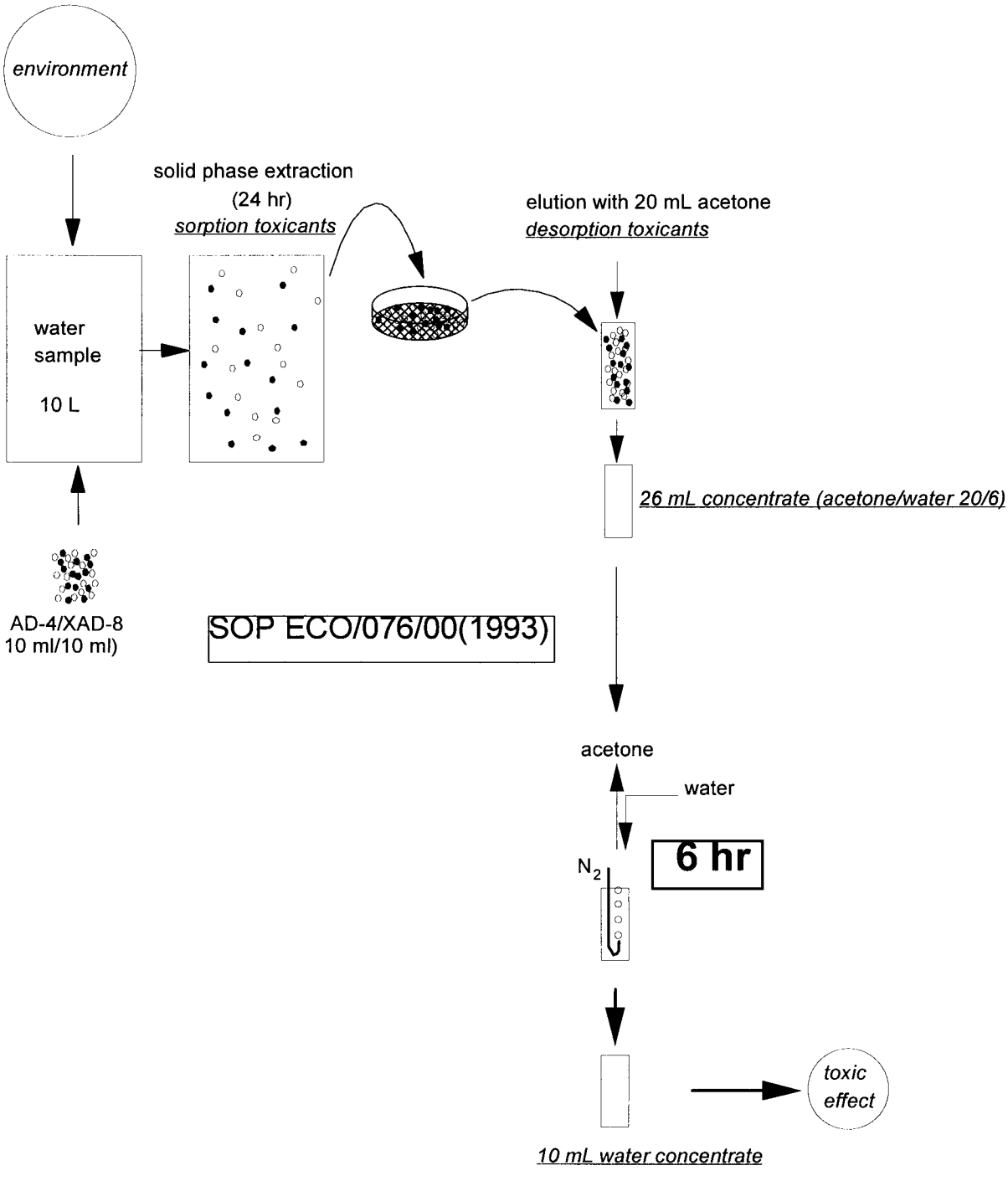


Figure 1. Schematic representation of the procedure, according to SOP ECO/076 (1993), to extract organic toxicants from a water sample and to concentrate them, applied by De Zwart & Folkerts (!990).

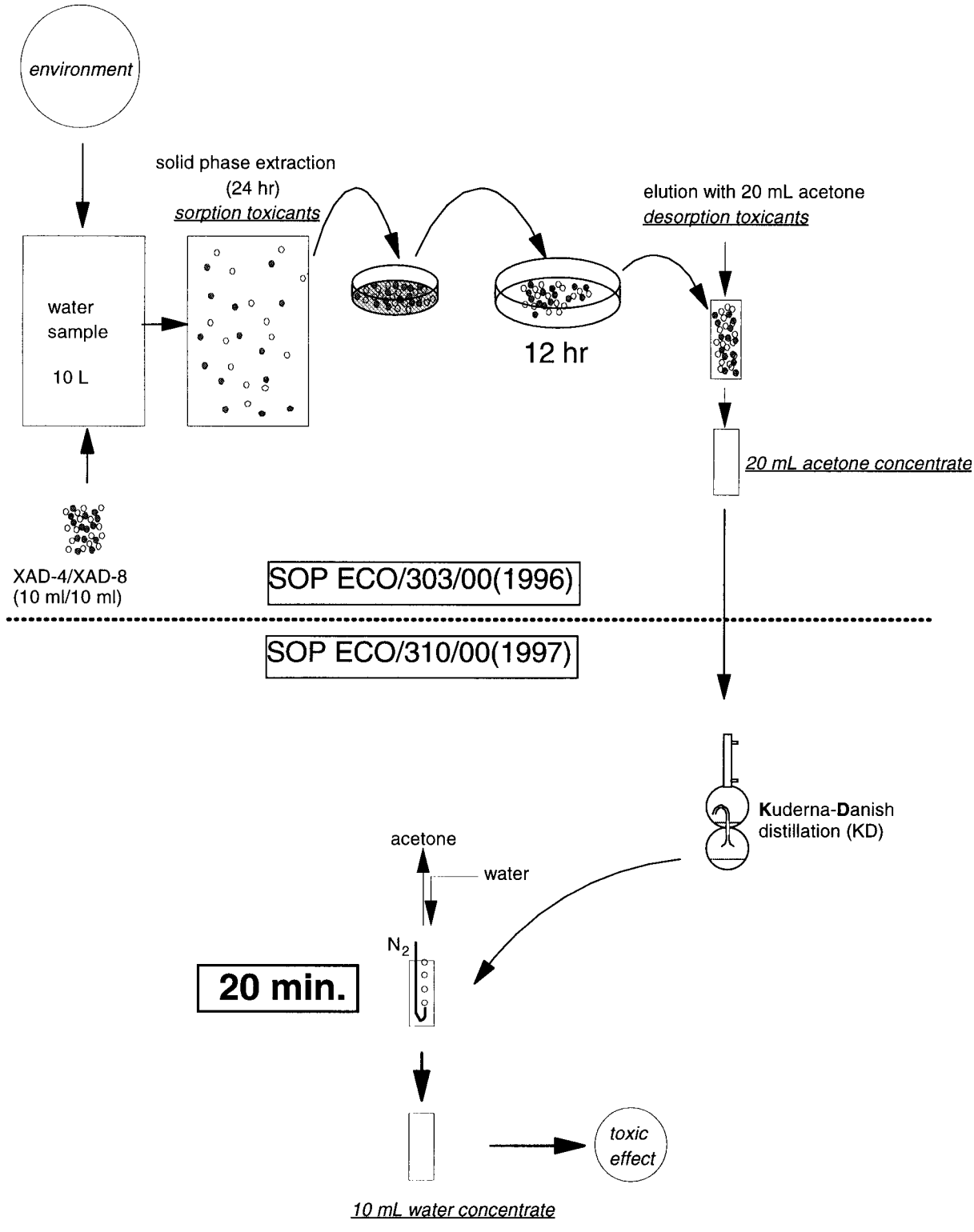


Figure 2. Schematic representation of the procedure to extract organic toxicants from a water sample and to concentrate them, according to a Kuderna Danish modification (De Zwart, Roghair & Struijs, 1996).

3.4 Super-critical fluid extraction (SFE)

A third concentration procedure is based on solid phase extraction according to SOP ECO/303 and supercritical fluid (CO_2) extraction of the organic compounds from XAD (see Figure 3). For technical reasons, pentachloroethane was omitted in the test mixture of narcotic compounds. Dried XAD was transferred into a 10 mL extraction vessel of the SFE apparatus and submitted to supercritical CO_2 extraction. Both the static and dynamic mode were applied to quantitatively release the chemicals from the XAD and to transfer them to the restrictor, where supercritical conditions are changed into atmospheric pressure. The restrictor is connected to an empty vessel (Accutrap) where the organic chemicals are trapped at -50 °C. From the start of the SFE extraction the trap vessel will gradually become packed with ice crystals during the extraction process. This may have either a positive or a negative effect depending on the amount of ice per trap tube volume: it may enhance the trap efficiency or it may cause clogging the system. The amount of XAD (10 mL) still contains at least 1 mL of water, despite the overnight drying process according to SOP ECO 303. Therefore it is likely that the cold trap vessel contains this amount of water[3]. Subsequently, the trap was rinsed with hot water (75 °C). Rinsing water was collected (maximum 10 mL total per amount XAD used for 10 L water sample) via a needle in a crimp sealed sample vial (20 mL) in an ice bath.
Further details like pressure, temperature, flow rate of the super critical fluid as well as the application of both the static and the dynamic mode in conducting the SFE technique are given in Annex C.
The pesticide mixture was not included in these preliminary experiments. The aim was to improve the methodology for concentrating water rather than to validate the procedure on the basis of chemical recovery.

[3] This was confirmed in control experiments. After super-critical CO_2 extraction of the XAD was completed, the temperature of the Accutrap was brought to room temperature. A few drops of water could be observed.

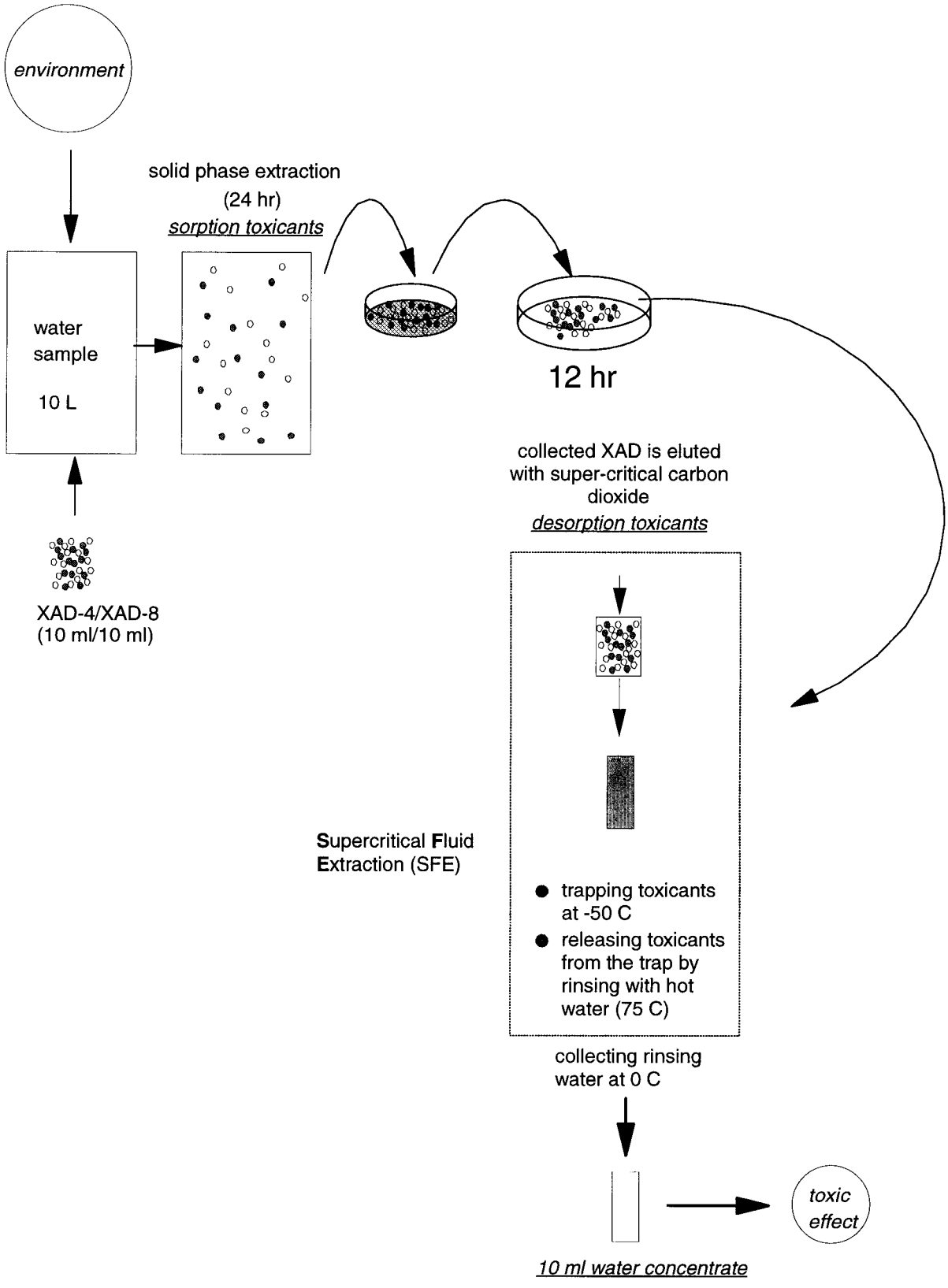


Figure 3. Schematic representation of a the SFE modification of the procedure, to extract organic toxicants from a water sample and to concentrate them (De Zwart, Roghair & Struijs, 1996).

4. RESULTS

Framed numbers in Figure 4 indicate the different stages where samples were taken in two standard procedures (in the following text and tables these numbers are referred to as numbers within brackets). Solid phase extraction was validated by measuring the concentration of test chemicals in water before (1) and after (2) the extraction step. The XAD was collected according to either SOP ECO/076 (without drying the resins) or SOP ECO/303 (major loss of water due to overnight drying), before transfer to the elution column. Concentrations in one bed volume of acetone - (3) and (4) - were measured to compare both SOPs. To evaluate the desorption process concentrations were also determined after two and three bed volumes of acetone. The final recovery of SOP ECO/076 after 6 hr purging was evaluated in 10 mL water concentrate (5), whereas the Kuderna-Danish modification (SOP ECO/310) was assessed from concentrations in the residue immediately after the KD-distillation (6) and in the final result after 20 min. purging with nitrogen and adjustment of the volume to 10 mL (7).

4.1 Recovery after solid phase extraction and acetone elution

4.1.1 The narcotic mixture

The measured concentrations in the surrogate water sample before XAD was added (1) are set to 100% (Table 1). The low residual percentages (2) in water illustrate that for all components of the narcotic mixture the partition is highly in favor of the XAD resin mixture. Solid phase extraction under these conditions is almost complete and residues in water are less than 5%. Apparently, for hydrophobic chemicals with log kow > 2, the amounts of XAD-4 and XAD-8 per volume water sample, the mode and duration of XAD-water contact are adequate. Recoveries in acetone (3) and (4) are given in the last two columns. The recovery after collection of 26 mL elutate (of which 6 mL is water), according to SOP ECO/076 is typically in the range of 80 to 100%. Percentages in the range of 60 and 80% were found for SOP ECO/303, but increased to 80 to 100% at a higher elution volume(Table 2). Approximately 3 bed volumes of acetone is required to reach a recovery as high as after only one bed volume in SOP ECO/076. These observations are summarized as follows:

1. solid phase extraction is a satisfactory procedure for these chemicals;
2. evaporating water from the XAD during a one night drying, according to SOP ECO/303, does not cause significant disappearance of the test compounds;
3. the test compounds are less easily desorbed after drying the XAD, according to SOP ECO/303.

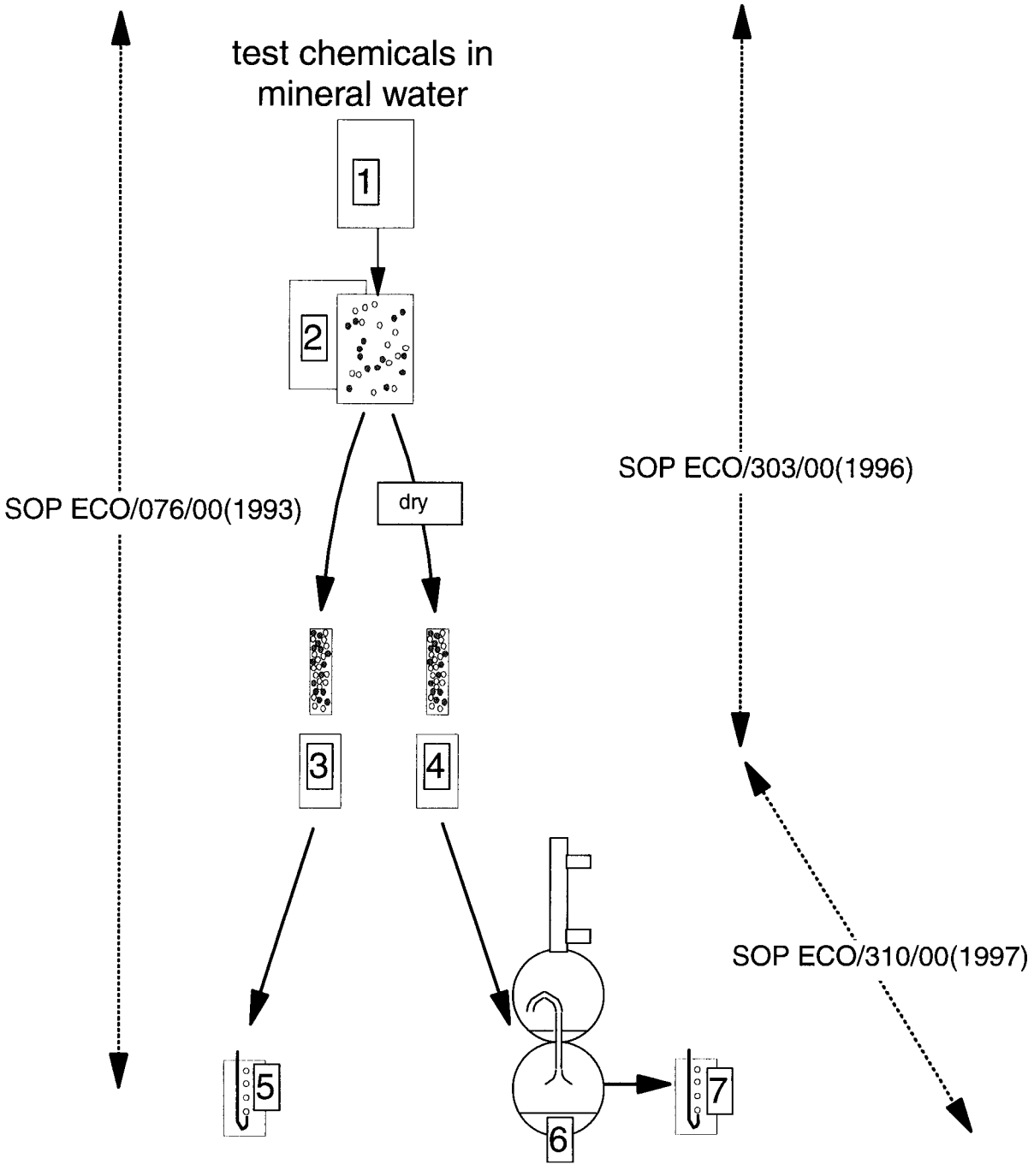


Figure 4. Sample sites (marked as framed numbers) of chemical recovery determinations.

Table 1. Recovery of narcotic test compounds after SPE and elution with (one bed volume) acetone.

narcotic mixture	(1) concentration (µg/L) in Spa Blauw		(2) residual % in Spa Blauw[1]	(3) % recovery[2] in acetone SOP ECO/076 (wet)	(4) % recovery[3] in acetone SOP ECO/303 (dry)
	added	measured[1]			
pentachloroethane	17.7	18.4 ± 1.1	3	95 ± 12	70 ± 9
1,4-dichlorobenzene	10.1	9.0 ± 0.4	0	96 ± 3	64 ± 6
hexachloroethane	0.9	0.9 ± 0.06	3	99 ± 5	59 ± 6
1,3,5-trichlorobenzene	2.0	1.7 ± 0.1	1	110 ± 6	73 ± 7
3,4-dichlorotoluene	4.0	4.8 ± 0.3	0	84 ± 3	60 ± 6
1,2,3-trichlorobenzene	3.9	3.6 ± 0.1	2	93 ± 3	61 ± 4
3-chloronitrobenzene	15.9	15.4 ± 0.9	2	81 ± 4	63 ± 9
2,4-dichloroaniline	44.1	45 ± 1.8	2	108 ± 8	78 ± 9
1,2,3,4-tetrachlorobenzene	2.2	1.7 ± 0.1	1	94 ± 6	64 ± 6
3,4-dichloro-nitrobenzene	15.0	15.2 ± 0.5	2	83 ± 7	61 ± 9
2,4,6-trichloroaniline	15.9	15.2 ± 0.7	2	94 ± 6	63 ± 5
pentachlorobenzene	0.6	0.5 ± 0.02	1	93 ± 6	62 ± 4

[1] n = 12, average ± standard deviation; [2] 26 mL acetone eluate contains 6 mL water (n = 6); [3] 20 mL acetone eluate (n = 6)

Table 2. SOP ECO/303: cumulative recoveries after several bed volumes of acetone

narcotics mixture	% recovery one bed volume	% recovery two bed volumes	% recovery three bed volumes
pentachloroethane	70 ± 9	73 ± 9	75 ± 8
1,4-dichlorobenzene	64 ± 6	81 ± 6	91 ± 4
hexachloroethane	59 ± 6	80 ± 7	91 ± 6
1,3,5-trichlorobenzene	73 ± 7	93 ± 5	104 ± 3
3,4-dichlorotoluene	60 ± 6	76 ± 6	85 ± 5
1,2,3-trichlorobenzene	61 ± 4	81 ± 4	91 ± 3
3-chloronitrobenzene	63 ± 9	80 ± 9	88 ± 6
2,4-dichloroaniline	78 ± 9	97 ± 9	108 ± 7
1,2,3,4-tetrachlorobenzene	64 ± 6	88 ± 6	99 ± 4
3,4-dichloronitrobenzene	61 ± 9	79 ± 7	88 ± 6
2,4,6-trichloroaniline	63 ± 5	82 ± 3	92 ± 3
pentachlorobenzene	62 ± 4	85 ± 4	98 ± 3

4.1.2 The pesticide mixture

Better performance of SOP ECO/076 compared to SOP ECO/303 was also found for the pesticide mixture (Table 3). However, results are more difficult to interpret than for the narcotic mixture. All components but one disappeared from the water phase (2). Two third of bentazone remained in the water, one third reached the sorbed phase as can be concluded from the recovery in acetone eluate (3). Bentazone, diuron and lindane showed more than 75 % desorption in SOP ECO/076. Desorption efficiency for bentazone, however, was very low after drying the XAD (4), even after 3 bed volumes of acetone (Table 4).
Recovery of chlorfenvinphos and fenchlorvos seems too low (<30%), whereas dichlobenil has a too high recovery. Artifacts in the analytical procedures can only be part of the explanation. Results after multiple bed volumes of elution solvent suggest that chlorfenvinphos is traced back to a level as high as 78 % in SOP ECO/303 (Table 4), but - surprisingly - not after 3 bed volumes acetone in SOP ECO/076 (data not shown). In the latter procedure recovery increased only from 25 to 28 % which is contrary to the trend of a higher sorption reversibility in SOP ECO/076. Also fenchlorphos deviates from this pattern. After 3 bed volumes in SOP ECO/076, the recovery remained zero (data not shown), whereas in SOP ECO/303 recovery after one bed volume of acetone was also zero, but increased to a constant level of 47 % after two bed volumes (Table 4).
Diuron, lindane and parathion-methyl are in the range of 70 - 100 % for SOP ECO/076 but in the range of 50 to 80 % if SOP ECO/303 is applied and 70 to 100 % after a higher elution volume, which seems in line with the narcotic mixture. The value of dichlobenil (104±25%) is suspect in view of the recovery obtained with SOP ECO/076 (152±29%), but also in relation to measured recovery in water (section 4.2, Table 6).

Table 3. Recovery of pesticides after solid phase extraction and elution with acetone

pesticides mixture	(1) concentration (µg/L) in Spa Blauw		(2) residual % in Spa Blauw[1]	(3) % recovery[2] in acetone SOP ECO/076	(4) % recovery[3] in acetone SOP ECO/303
	added	measured[1]			
bentazone	10.1	9.9±0.3	64±4	28±4	1.4±0.6
chlorfenvinphos	11.5	10.1±2.1	0	25±20	22±26
dichlobenil	2.1	1.6±1.0	0	152±29	104±25
diuron	9.7	9.3±0.7	0	103±17	78±6
fenchlorphos	1.0	0.6±0.3	0	0	0
lindane	2.0	1.4±0.4	0	95±6	58±11
parathion-methyl	10.5	9.7±1.3	0	68±16	46±12

[1] n = 11; [2] 26 mL acetone eluate which contains 6 mL water (n = 6); [3] 20 mL acetone eluate (n = 6)

Table 4. SOP ECO/303: cumulative recoveries in acetone

pesticides mixture	% recovery one bed volume	% recovery two bed volumes	% recovery three bed volumes
bentazone	1.4±0.6	1.7±0.9	2.8±1.6
chlorfenvinphos	22±26	78±30	78±31
dichlobenil	103±17	128±26	128±26
diuron	78±6	98±7	99±7
fenchlorphos	0	47±9	47±9
lindane	58±11	82±12	82±12
parathion-methyl	46±12	71±13	71±13

4.2 Recovery after removal of acetone

4.2.1 The narcotic mixture

In Table 5 the average of three recovery percentages relative to measured concentrations in the surrogate water samples are given. Columns indicated by (5) and (7) display overall recoveries of the two methods to be compared.

Table 5. Recovery of the narcotic mixture in water

narcotics mixture	(5) % recovery in water concentrate SOP ECO/076	(6) % recovery after KD distillation SOP ECO/310	(7) % recovery after KD distillation; 20 min purging SOP ECO/310
pentachloroethane	0	3±3	0
1,4-dichlorobenzene	0	44±1	0
hexachloroethane	0	49±13	0
1,3,5-trichlorobenzene	0	48±3	0
3,4-dichlorotoluene	0	41±2	0
1,2,3-trichlorobenzene	0	49±3	0
3-chloronitrobenzene	0	60±7	49±2
2,4-dichloroaniline	4	61±7	49±7
1,2,3,4-tetrachlorobenzene	0	48±3	0
3,4-dichloro-nitrobenzene	0	58±2	48±2
2,4,6-trichloroaniline	0	52±6	44±2
pentachlorobenzene	0	46±4	6±6

Concentrations of the test compounds were very low or even below the detection limit, resulting in a very poor recovery for SOP ECO/076 (5). This procedure is not appropriate to cover compounds with H > 0.1 Pa m^3 mol^{-1}. If 20 minutes of purging could be avoided, SOP ECO/310 would cover all test compounds except pentachloroethane (6). Unfortunately, the whole procedure seems only appropriate for the 4 test compounds with a polar functional group and to a minor extent for pentachlorobenzene.

4.2.2 The pesticide mixture

Results for chlorfenvinphos and dichlobenil are too high and certainly due to artifacts. Recovery of chlorfenvinphos in acetone was as low as 22 -25% in both procedures (in SOP ECO/076 even after 3 bedvolumes) and dichlobenil again exceeds 100 %. SOP ECO/076 is rather successful if bentazone, diuron and parathion-methyl is concerned. Zero recovery for fenchlorphos is in accordance to the failure to desorp this pesticide from XAD. Lindane is too volatile for SOP ECO/076, but 1/6 of it could be found in the water concentrate when the KD modification was applied, according to the trend of the narcotic mixture.
The lower recoveries of bentazone, diuron and parathion-methyl in the KD modification is entirely attributed to the lower recovery in SOP ECO/303.

Table 6. Recovery of the pesticide mixture in water

pesticide mixture	(5) % recovery in water concentrate SOP ECO/076	(6) % recovery[2] after KD distillation SOP ECO/310	(7) % recovery[3] after KD distillation; 20 min purging SOP ECO/310
bentazone	**28±3**	1±1	**8±0.4**
chlorfenvinphos	**95±13***	51±7*	**42±15***
dichlobenil	**198±57***	106±15*	**100±25***
diuron	**91±2**	55±3	**45±4**
fenchlorphos	**0**	0	**2±0.4**
lindane	**0**	27±11	**16±0.4**
parathion-methyl	**79±8**	46±6	**40±13**

* non-reliable results, probably due to analytical artifacts

4.3 SFE modification: preliminary results

Only the narcotic mixture without pentachloroethane was submitted to this alternative method. Because earlier experiences proved the high efficiency of solid phase extraction for the narcotic mixture, only concentrations denoted by (3) were measured (Figure 5).

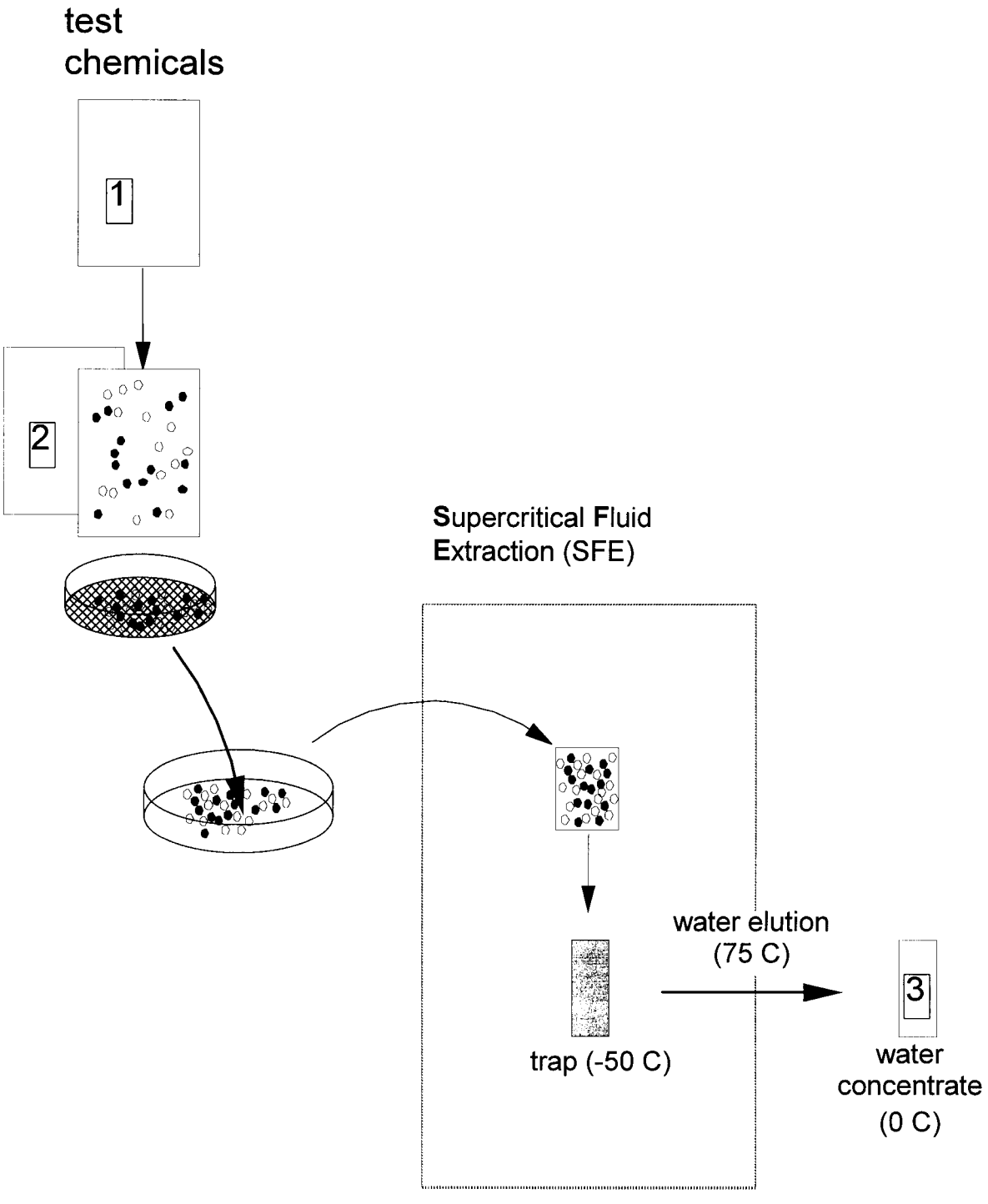


Figure 5. Sample sites of chemical recovery determinations.

Results summarized by Table 7 showed a strong improvement compared to the standard procedures that require evaporation of acetone. Results lower than 50% could not be attributed to incomplete extraction from XAD by super-critical CO_2. To verify this, XAD was removed from the extraction vessel and submitted to 10 bed volumes acetone elution; only minor amounts of the test chemicals could be found (data not shown).
Recovery is surprisingly high for the very hydrophobic chemicals tetra- and pentachlorobenzene. These chemicals are trapped at -50 °C and probably are partly sorbed onto ice crystals. Elution of these very lipophilic chemicals with hot water is almost complete as could be concluded from the very low concentrations in acetone which was used to rinse the trap afterwards. These measurements (data not shown) tentatively indicate that reduced recoveries should be ascribed to a failure to completely trap some chemicals.

Table 7. Recovery of the narcotic mixture after super-critical CO_2 extraction and trapping at - 50° C.

narcotic mixture (without penta chloroethane)	(3) % recovery in water concentrate
1,4-dichlorobenzene	**3**
hexachloroethane	**5**
1,3,5-trichlorobenzene	**22**
3,4-dichlorotoluene	**25**
1,2,3-trichlorobenzene	**31**
3-chloronitrobenzene	**51**
2,4-dichloroaniline	**51**
1,2,3,4-tetrachlorobenzene	**52**
3,4-dichloro-nitrobenzene	**60**
2,4,6-trichloroaniline	**57**
pentachlorobenzene	**49**

5. DISCUSSION, CONCLUSIONS AND RECOMMENDATION

The capability to accumulate organic toxicants in water have been tested by three different methods. The aim was a method that provides a 1000-fold water concentrate that is suitable as the starting material for testing the toxicity for aquatic organisms. From the resulting EC^f_{50} or NEC^f the effect parameter PAF(measured) is evaluated. It was anticipated that a concentration factor as high as 1000 is necessary to sufficiently span the exposure range for obtaining EC^f or NEC^f.

5.1 SOP ECO/076

Substances with a very low volatility can be isolated from surface water and almost quantitatively transferred to a water volume that is orders of magnitude smaller. Some pesticides may be concentrated in this way, others not or only to a low extent. Parathion-methyl and diuron have been accumulated in water almost 1000-fold, but bentazone and probably also chlorfenvinphos at least 100-fold. If SOP ECO/076 allows concentrating chemicals with comparable physico-chemical properties, at least part of the unknown pesticide cocktail in surface water is expected to be accumulated.
Although the results reported here should not be appreciated as a comprehensive validation of SOP ECO/076, the data may contribute to the plausibility of measured differences in toxicity in River Rhine water concentrates, reported by De Zwart & Folkerts (1990). Differences in toxic loading by pesticides could have been the causal relationship.
The method, however, does not seem suitable for the halogenated hydrocarbons found during "Fliessende Welle" surveys of the River Rhine (Stachel et al., 1994). Most components of the narcotic test mixture are typical for this class of water pollution (IAWR, 1982). Therefore, it should be assumed that biomonitoring based on SOP ECO/076 does not cover this segment of toxicity.

5.2 SOP ECO/303

According to SOP ECO/076, the eluate volume was 1.3 times the bed volume because freshly sieved XAD contains approximately 0.3 bed volume water. Most of this water is lost if, according to SOP ECO/303, the XAD is allowed to stay overnight in a open petri disc under a gentle air flow. The last method is preferred - if not required - if water has undesirable effects on subsequent manipulations. KD-distillation is more efficient if the water content is lower and super-critical CO_2 extraction applied to XAD would fail if XAD contains too much water that is released from the extraction vessel. Water causes clogging inside or in the vicinity (tubing) of the cold trap due to ice forming. Preliminary experiences indicate that careful application of SOP ECO/303 is a prerequisite to apply the super-critical CO_2 technique.
SOP ECO/303 seems a suitable procedure to isolate toxicants from a water sample. The sorbed toxicants, however, are less easily eluted from the dried XAD if acetone is used compared to SOP ECO/076.

5.3 SOP ECO/310

The KD distillation is a satisfactory method to evaporate acetone until the test chemicals are concentrated in a residue that merely consists of water but unfortunately still contains a too high amount of acetone. If the presence of some residual acetone were no limiting factor, the procedure would be adequate because almost all test chemicals are still present at a satisfactory level. Unfortunately, adjusting the volume of the residue with a small amount of water - up to 10 mL - until a 1000-fold concentration is achieved, does not decrease the acetone concentration to an acceptable level. Removing the acetone by purging - even for a relatively short period compared to SOP ECO/076 - still appears the most critical step in the whole procedure. Most of the hydrophobic chemicals are volatilized during 20 min. of purging with nitrogen. Considering the high recovery of most chemicals *before 20 min purging*, it is interesting to investigate the possibility to dilute the residue by a factor 10 more and to accept only a 100-fold concentration. Perhaps this would allow to cancel the purging step.

5.4 SFE modification

Although several problems have to be resolved, in particular the consistency of operation of the SFE apparatus, the SFE modification seems full of promise. It is an improvement compared to the KD modification because compounds with H values up to 1000 Pa m^3 mol^{-1} are concentrated (Figure 6). Moreover, the method is less cumbersome because the combination of elution, distillation and purging is replaced with one instrumental manipulation. Further research is needed to test the stability of operation and to evaluate the reliability of the method. This is being conducted in the framework of the project "pT Total Extraction Water".

5.5 Relationships between chemical recovery and a physico-chemical property

5.5.1 Hydrophobicity

With the exception of bentazone, all test chemicals are rather hydrophobic with log K_{ow} higher than 2.6 (3-chloronitrobenzene). Sorption of these chemicals on the XAD-4/8 resin mixture is virtually complete. The percentage sorbed was low for bentazone and probably associated with its low hydrophobicity (log K_{ow} = -0.46 at pH 7). It is recommended in further research to include some test chemicals in the log K_{ow} range of -0.5 and 2.5. Moreover, most pesticides that have been measured in surface water, belong to this hydrophobicity range.

5.5.2 Henry's law constant

The combination of SOP ECO/303 and SOP ECO/310 seems an improvement with respect to SOP ECO/76 for chemicals with Henry's law constants (H) higher than 0.1 Pa m^3 mol^{-1}. In Figure 6 the recovery of the narcotic substances is plotted versus H on a logarithmic axis. It is clear that significantly more chemicals are recovered when the KD modification is applied. Figure 6 does not give information below which value of H significant recovery levels can be achieved when SOP ECO/076 is applied, but it clearly demonstrates that there is a range of at least three orders of magnitude in H where the KD-version produces significant recoveries, where complete loss of substances is observed in SOP ECO/076.
Nevertheless, some pesticides with lower H values were recovered with high efficiency by SOP ECO/076. Diuron (91%), chlorfenvinphos (95%), betanzon (28%[4]) and parathion-methyl (79%) fall in the range below 0.001 Pa m^3 mol^{-1}. Unfortunately, neither the narcotic nor the pesticide mixture contains chemicals in the range of 0.001 to 0.1 Pa m^3 mol^{-1}. There is no information more specific than that somewhere in this range the recovery according to SOP ECO/076 decreases from 100% to 0%. Nevertheless, we believe that it is unlikely that in this procedure chemicals with H higher than 0.1Pa m^3 mol^{-1} can be concentrated. The recovery of dichlobenil (198%) as such is suspect, but also if the relatively high volatility (H = 0.8 Pa m^3 mol^{-1}) is considered. Therefore, we tentatively conclude that results are erroneous, due to artifacts.

5.5.3 Vapour pressure

A more consistent pattern is obtained if the recovery percentage is plotted versus the vapour pressure (see Figure 7). The cut-off value for the SFE modification is clearly 100 Pa, whereas significant recoveries (20 - 30%) were found for 3 chemicals in the range of 30 - 40 Pa.
A plateau is reached below 1 Pa whereas at 10 Pa still 2/3 of this plateau level is reached. 3,4-Dichloronitrobenzene has the lowest vapour pressure in the series (0.04 Pa), but could not be detected in the water concentrate prepared according to SOP ECO/076. Therefore it is not surprising that for fenchlorphos with P = 0.01 also zero recovery (not shown in Figure 7, see Table 4) was found.

[4] This low percentage is due to the low efficiency of sorption onto XAD, probably due dissociation. With respect to the amount sorbed on XAD, the desorption efficiency is comparable to diuron, chloorfenvinfos and parathion-methyl.

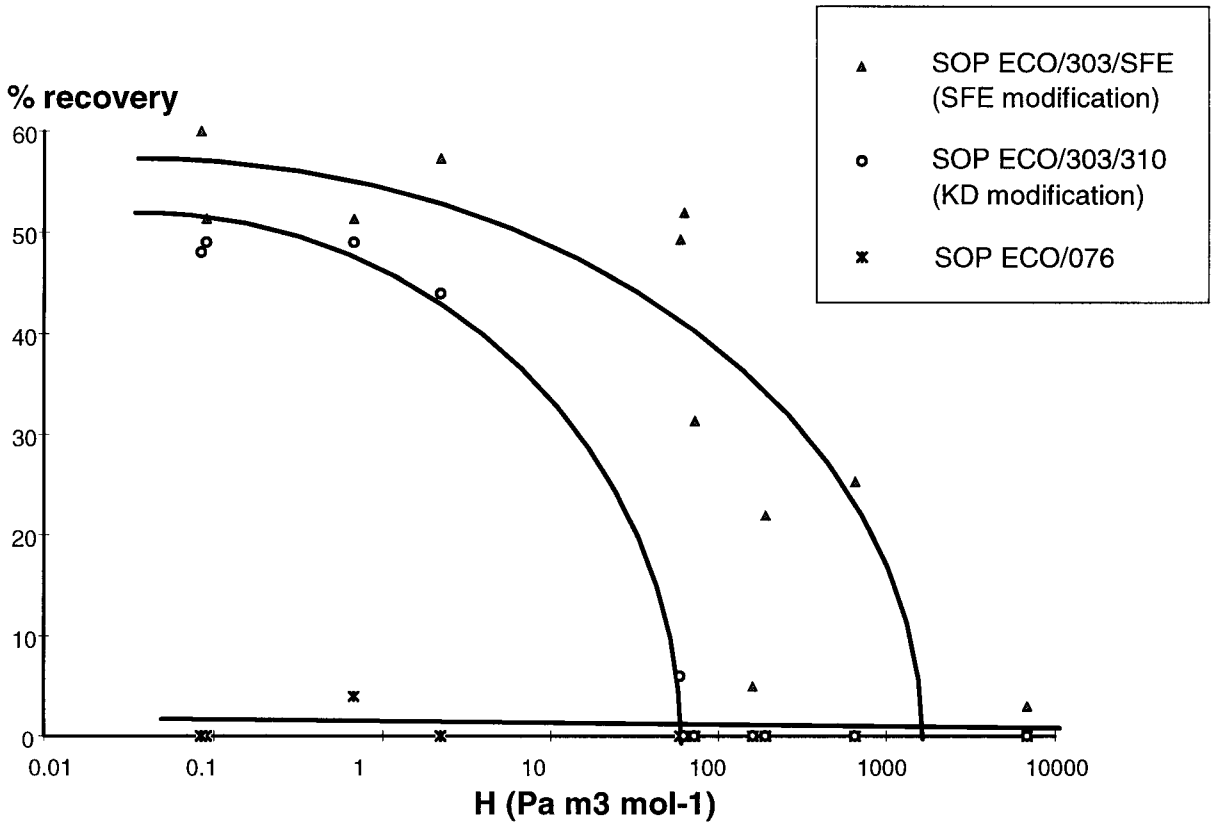


Figure 6. Chemical recovery vs Henry's law constant (H) plot for the narcotic test mixture.

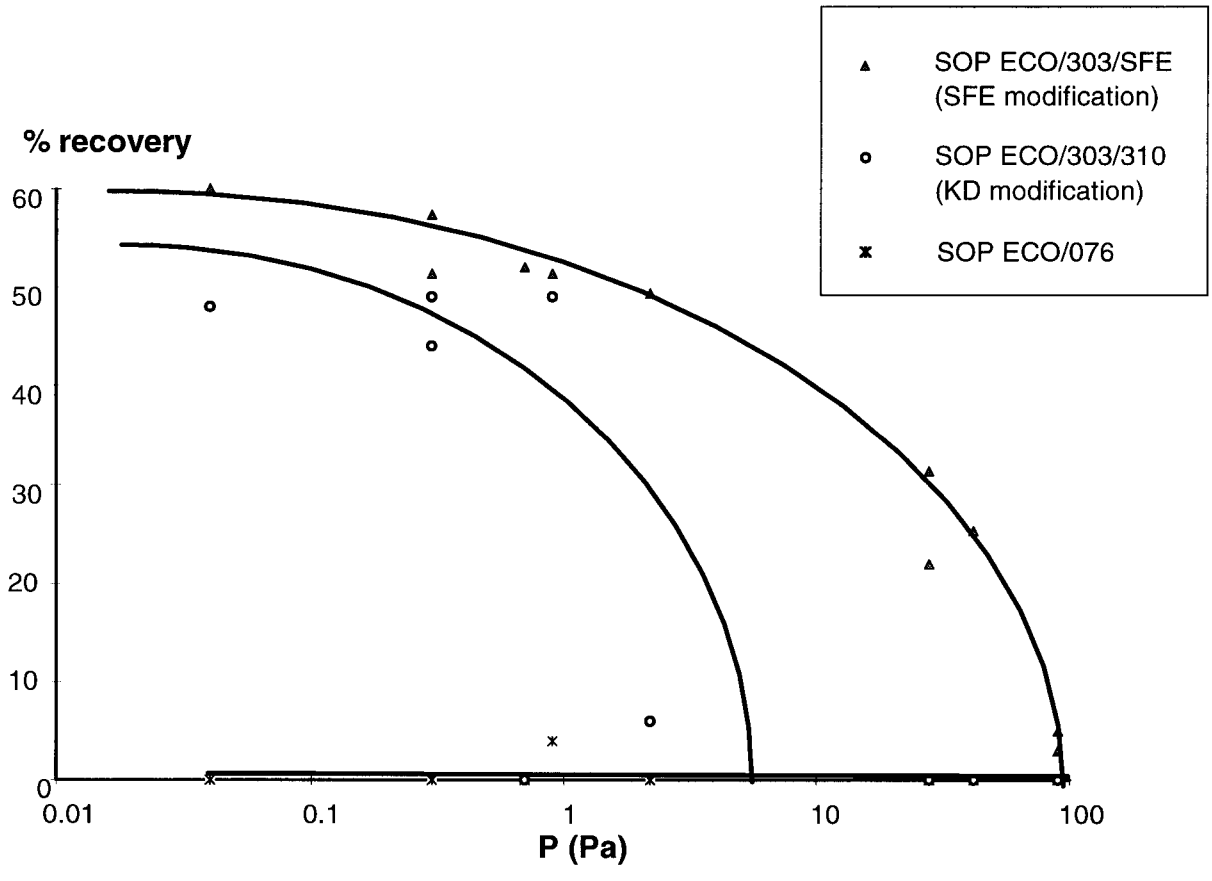


Figure 7. Chemical recovery vs vapour pressure plot for the narcotic test mixture.

5.6 Future research

If we consider that approximately 100 mL of concentrate is necessary for the array of toxicity testing with the currently available techniques, it is clear that monitoring would be very cumbersome. Sampling and especially processing 100 L water is very laborious and it claims much laboratory capacity. It also produces high volumes of chemical waste (acetone and other solvents) unless supercritical fluid extraction can be applied, not only to prepare water concentrate but also to purify XAD resins. If the recovery of toxicants could be improved to such an extent that a factor 100 is adequate for characterising aquatic toxicity, the applicability would be enhanced.
The use of super-critical carbon dioxide to release the toxicants from an adsorbent offers an opportunity to concentrate toxicants in a more efficient way. Achieving a concentration factor not higher than 100 would strongly increase the applicability of the concentration procedure for biomonitoring.

LITERATURE

ASTER (1994), ASsessment Tools for the Evaluation of Risk, Update June 1, 1994, US-EPA, Duluth, MN, USA

De Groot, A.C., R.E. van de Kamp and W.J.G.M. Peijnenburg, 1996. Ontwikkeling en validatie van een analysemethode voor de bepaling van 12 gehalogeneerdekoolwaterstoffen geaddeerd aan SPA-blauw water. ECO-notitie 96-02.

De Zwart, D and A-J Folkerts, 1990. Monitoring the toxicity of organic compounds dissolved in Rhine water. Hydrobiol. Bull. 24 (1), 5-12.

De Zwart, D. and H.J.G. Polman, 1993. De toxiciteit van Maas- en Rijnwater in 1992. Rapportnummer 719102 023, Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven.

De Zwart, D. and H.J.G. Polman, 1994a. Toxiciteitsmetingen m.b.v. de Microtox-methode aan oppervlaktewater en oppervlaktewaterconcentraten van de Maas in 1993. Rapportnummer 607042 002, Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven

De Zwart, D. and H.J.G. Polman, 1994[b]. Metingen in het kader van het Biologisch Monitoring Programma van RWS/RIZA naar de toxiciteit van oppervlaktewater-concentraten van rijkswateren in 1993. Rapportnummer 607042003. Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven.

De Zwart, D., Roghair, C.J. and Struijs, J. (1996). De pT-methode voor het bepalen van milieutoxiciteit, Noodzaak en ontwikkelingsstatus. RIVM-Rapport nr. 607042 007.

Hendriks, A.J., Maas-Dieoeveen, J.L., Noordsij, A, Van der Gaag, M.A. (1994). Monitoring response of XAD-concentrated water in the Rhine delta: a major part of the toxic compounds remains unidentified. Wat. Res. 28, No 3, 581-598.

IAWR (1992). Internationale Arbeitsgemeinschaft der Wasserwerke im Rheineinzugsgebiet, Jahrgang 1982.

Noordsij, A. (1996). Organische stoffen in (drink)water. Evaluatie van 15 jaar VEWIN-onderzoek. SWE 96.003 VEWIN/KIWA rapport.

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 002

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.

SOP LOC/123 (1991). De bepaling van organofosforverbindingen in water.

Slooff, W., Bremmer, H.J., Hesse, J.M., and Matthijsen A.J.C.M. (eds.) (1991). Integrated Criteria Document Chlorobenzenes, RIVM Report no. 710401015.

Struijs, J. and Van Buren, L. (1995) Milieutoxiciteit (pT) in water (I): het opwerken tot waterconcentraten. RIVM-Rapport nr. 607042 006.

Stachel, B., Lahl, U. and Zeschmar, B. (1984). Pollution of the River Rhine with halogenated organic compounds, An investigation during a "Fliessende Welle". The Science of the Total Environment **40**, p 103-113.

Ten Hulscher, Th. E.M., Van der Velde, L.E., and Bruggeman, W.A. (1992) Temperature Dependence of Henry's Law Constants for Selected Chlorobenzenes, Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons. Environ. Tox. & Chem. **11**, 1595-1603.

Tomlin, C. (Ed.), 1994. The Pesticide Manual (A World Compendium), Tenth Edition, Crop Protection Publications.

Van de Broek, H. (1990), Kuderna-Danish destillatie-opstelling, Laboratorium Praktijk, maart 1990, 95.

Vaal, M.A. and A.-J. Folkerts (1997) Microbiotests: sensitivity and usefulness. RIVM-Report nr. 607042 009

Van Egmond, N.D., De Mik, G., Eijsackers, H., De Zwart, D., J. Struijs, Roghair, C.J. and Notenboom, J. (1996). Strategic recommendations by the directory board of RIVM, dd 13 Nov 1996.

Wolfe, M.F., Hinton, D.E., Seiber, J.N. (1995). Aqueous sample preparation for bioassay using supercritical fluid extraction. Environ. Toxicol. and Chem., 14, 1001-1009.

ANNEX A AUXILARY SUBSTANCES AND EQUIPMENT

Adsorbents used for solid phase extraction

XAD-4 is a cross-linked polystyrene-divinylbenzene (Fig A1) and used as a non-polar macro-reticular resin of certain physical properties (see Table A1). XAD-8 is a polymethylmethacrylate resin (Fig A2), is more polar and has a wider pore diameter.

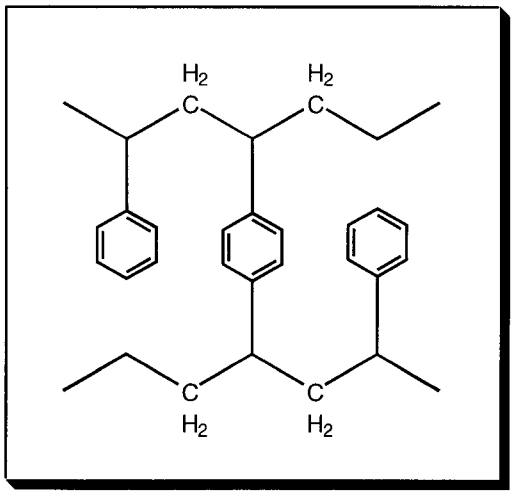


Fig A1

polystyrene divinylbenzene co-polymer

XAD-4

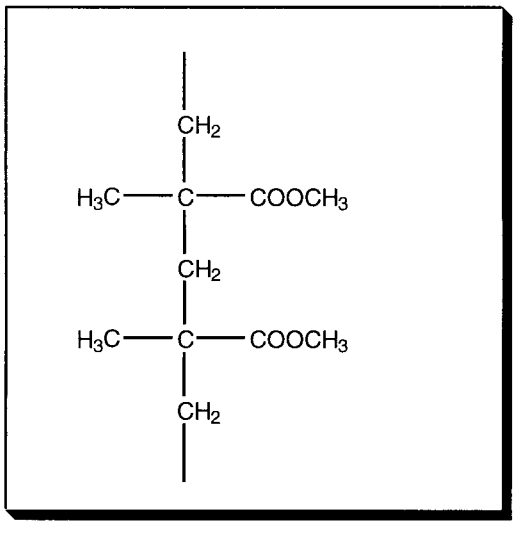


Fig A2

polymethylmethacrylate

XAD-8

Table A1. Properties of the used XAD resins

Type XAD	Porosity (vol %)	Surface ($m^2 g^{-1}$)	Pore diameter (Å)
XAD-4	51	780	50
XAD-8	52	140	240

XAD-4 was obtained from Rohm & Haas, Antwerp, Belgium and XAD-8 (official name: DAX-8) from Applied Science Group, Emmen. XAD resins, supplied by the manufacturers, are expected to contain impurities as a result of the production proces. Prior to use, the XAD was treated in order to prevent contamination of the water sample by those impurities. KIWA purified the XAD resins according to an automatic procedure which is summarized as follows (for more details the reader is referred to Noordsij, 1996):

- Washing with 4% sodium hydroxide solution, repeated 10 times
- Washing with 4% hydrochloric acid, repeated 10 times
- Washing with twice distilled de-ionised water, repeated 10 times
- Washing with methanol, repeated two times
- Soxhlet extraction with hot methanol for 24 hours
- Washing with ethanol, repeated 3 times
- Soxhlet extraction (24 hr) with an azeotropic ethanol/cyclohexane mixture (30.5:69.5)
- Washing with twice distilled methanol, repeated 5 times

Clean XAD is stored in methanol, in the dark at room temperature. XAD shrinks when it is directly transferred from methanol to water or acetone. Particularly XAD-4 may shrink by approximately 20% and, as an unwanted side-effect, unknown toxic components may be released. These contaminants are removed by washing the resins prior to adding XAD4/8 to a water sample. The procedure deviates slightly from SOP ECO/076 (see also Struijs & Van Buren, 1995). A mixed slurry of both resins in methanol is transferred to a glass extraction column and washed in succession with:

- 2 bed volumes of methanol p.a.
- 2 bed volumes of acetone p.a.
- 2 bed volumes of methanol p.a.
- 12 bed volumes of Spa blauw.

Solvents

Methanol p.a. (washing XAD): Merck;
Acetone p.a. (elutions and washing XAD): Merck;
Acetone nanograde (preparation of stock solutions): Baker;
Dichloromethane p.a.(analysis pesticides): Baker
n-Hexane p.a. (analysis narcotic compounds): Baker.

Gases

Nitrogen was derived from the internal facilities of the institute ("5.0 quality"). CO_2 used for SFE was obtained from Scott Specialty Gases, SFC-grade.

ANNEX B TEST CHEMICALS

Properties of the test chemicals

Table B1. Trade mark and purity of the narcotic chemicals

compound	mol.formula	CAS nr	trade mark	purity
pentachloroethane	C_2HCl_5	76-01-7	RdHaën/Lancaster	98%
1,4-dichlorobenzene	$C_6H_4Cl_2$	106-46-7	Fluka/Merck	>99%
hexachloroethane	C_2Cl_6	67-72-1	Aldrich	99%
1,3,5-trichlorobenzene	$C_6H_3Cl_3$	108-70-3	Acros	99%
3,4-dichlorotoluene	$C_6H_3Cl_2OH$	95-750	Aldrich	97%
1,2,3-trichlorobenzene	$C_6H_3Cl_3$	87-61-6	Merck	>98%
3-chloronitrobenzene	$C_6H_4ClNO_2$	121-73-3	Janssen	98%
2,4-dichloroaniline	$C_6H_5Cl_2N$	554-00-7	Aldrich	97%
1,2,3,4-tetrachlorobenzene	$C_6H_2Cl_4$	634-662	Merck	>96%
3,4-dichloronitrobenzene	$C_6H_3Cl_2NO_2$	99-54-7	Janssen	95%
2,4,6-trichloroaniline	$C_6H_4Cl_3N$	634-93-5	Aldrich	97%
pentachlorobenzene	C_6HCl_5	608-93-5	Aldrich	98%

Table B2. Physicochemical properties of pesticides

mixture I (narcotics)	Henry's law constant (Pa m^3 mol^{-1})		water solubility (mg L^{-1})	vapour pressure (Pa)	boiling point (°C)	log k_{ow}
	calculated	measured[1]	20-25 °C	20-25 °C		
pentachloroethane[2]	1400		63.7	440	162	3.6
1,4-dichlorobenzene[3]	160		83	90	174	3.5
hexachloroethane[2]	6800		3.1	90	186	4.6
1,3,5-trichlorobenzene[3]	200	190	25	28	208	4.0
3,4-dichlorotoluene[2]	650		10.5	42	208	4.2
1,2,3-trichlorobenzene[3]	300	72	17	28	218	3.8
3-chloronitrobenzene[2]	0.09		501	0.3	235	2.6
2,4-dichloroaniline[2]	0.67		214	0.9	245	2.8
1,2,3,4-tetrachlorobenzene[3]	250	62	4.3	0.7	216	4.3
3,4-dichloronitrobenzene[2]	0.084		97	0.04	255	3.3
2,4,6-trichloroaniline[2]	2.2		26	0.3	262	3.6
pentachlorobenzene[3]	980	59	0.6	2.2	277	4.8

[1] Ten Hulscher et al. (1992); [2] water solubility, vapour pressure and k_{ow} from QSAR calculations, ASTER (1994); [3] water solubility, vapour pressure and k_{ow} derived from Slooff et al.(1991).

Table B3. Trade mark and purity of the narcotic chemicals

compound	mol.formula	CAS nr	trade mark	purity
bentazone	$C_{10}H_{12}N_2O_3S$	25058-89-0	Riedel de Haën	98%
chlorfenvinphos	$C_{12}H_{14}Cl_3O_4P$	330-54-1	Riedel de Haën	99%
dichlobenil	$C_7H_3Cl_2N$	1194-65-6	Riedel de Haën	99%
diuron	$C_9H_{10}Cl_2N_2O$	470-90-6	Riedel de Haën	98%
fenchlorphos	$C_8H_8Cl_3O_3PS$	299-84-3	Riedel de Haën	99%
lindane	$C_6H_6Cl_6$	58-89-9	Riedel de Haën	99%
parathion-methyl	$C_8H_{10}NO_5PS$	298-00-0	Riedel de Haën	99%

Table B4. Physicochemical properties of the pesticides (Tomlin, 1994).

mixture II (pesticides)	Henry's law constant ($Pa\ m^3\ mol^{-1}$)	water solubility ($mg\ L^{-1}$) 20-25 °C	vapour pressure (Pa) 20-25 °C	boiling point (°C)	mol weight ($g\ mol^{-1}$)	log k_{ow}
bentazone	$1.9\ 10^{-4}$	570	$4.6\ 10^{-4}$		240	- 0.5
chlorfenvinphos	$3.8\ 10^{-4}$	95	$1\ 10^{-4}$	167-170	361	3.2
dichlobenil	$8.4\ 10^{-1}$	18	$9\ 10^{-2}$	270	172	2.7
diuron	$6.1\ 10^{-6}$	42	$1.1\ 10^{-6}$		233	2.8
fenchlorphos	29	1.1	$1\ 10^{-1}$		323	5.3
lindane	$1.5\ 10^{-2}$	12.7	$5.6\ 10^{-4}$	323	349	3.8
parathion-methyl	$9.8\ 10^{-4}$	55	$2\ 10^{-4}$	154	269	3.4

ANNEX C TEST MIXTURES AND EXTRACTION METHODS

Preparation of test mixtures

Stock solutions
Of each test chemical a stock solution in acetone was made in an appropriate concentration. All stock solutions were stored at -20 °C. Prior to each experiment, stock solutions were allowed to adapt to room temperature and a mixture of these stock solutions in suitable ratio's was prepared (hereafter referred to as the mixture stock solution).

Surrogate water samples
10L reference water (Spa blauw, pH=7) was transferred to a 10 L borosilicate bottle. The water is spiked with 100 µL of the mixture stock solution using a microsyringe. The needle of the syringe was kept beneath the water surface while the mixture stock solution was administered. The bottle was gently agitated for several hours to allow the components to dissolve in water.

Solid phase extraction procedure

Scott-Duran borosilicate flasks, 5 or 10 L with cap and teflon inlay were used. Pipettes, petri dishes, extraction columns with sintered frits (porosity grade 0) fitted with teflon stoppers and other glassware were made of borosilicate. Bühler VKS 75/A, Bühler SM25 and Kera rotation/shaking equipment were utilized. 20 mL of the purified XAD was conditioned as described in Annex A and added to the surrogate water sample. In a dark room at 20°C the XAD was freely in contact with water by shaking or rolling the bottle. After twenty-four hours the XAD was collected on a 100 µm sieve.

Further manipulations after the solid phase extraction

Elution was according to 3.3.1. After elution the acetone samples were stored in 20 mL vials with crimp cap and teflon inlay (Chrompack) at 4 °C.

Kuderna-Danish distillation. A unit consists of a KD evaporator concentration vessel of 500 mL, a KD evaporator collection vessel of 500 mL; a graduated glass tube with glass stopper; Graham condensers; a tall 3L beaker; boiling chips (Merck) and pasteur's pipettes.

Supercritical Fluid Extraction (SFE). Suprex Prepmaster SFE apparatus was equipped with a variflow restrictor and a programmable accutrap. A stainless steel extraction vessel of 10 mL was used. The instrument was modified by replacing several tubings between the restrictor and the accutrap.
The following conditions were chosen:

- 15 min. static extraction at 340 atm. and 80 °C, trap at 5 °C

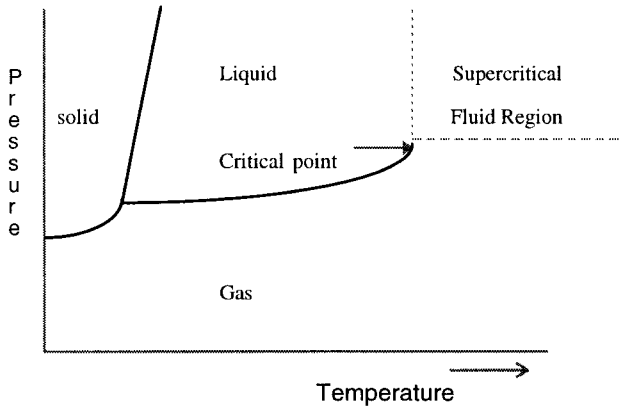
- 45 min dynamic extraction, 1 ml/min, 340 atm. and 80 °C, trap empty at -50 °C
- desorption from the trap at 75 °C with Spa Blauw 1ml/min

SFE applied to XAD was hampered by the effect of residual water. Generally, water may help or hinder the extraction process. The presence of water has been reported to both increase and decrease recoveries, depending on the system studied. Water can affect the mechanical performance of SFE by causing restrictor plugging (Bøwadt, 1995). The most obvious approach is to dry the XAD but this may lead to losses of even relatively non-volatile compounds. An alternative to drying samples is to mix the sample with a drying agent, but this can also lead to losses of volatile compounds, since drying agents can cause substantial heating of the sample. Moreover, the use of drying agents may lead to unwanted introduction of toxicants to the water sample. Therefore it was decided to accept a maximum amount of water which would not cause clogging. These condition were met if:

- XAD was dried according to SOP ECO/303;
- the original tubing between the restrictor and the accutrap was replaced with wider tubing, which was heated by a föhn during the dynamic extraction;
- the accutrap was empty at the start of the extraction

ANNEX D SUPER-CRITICAL FLUID EXTRACTION

At the critical temperature of a substance, its vapour and its liquid have the same density, and the substance is referred to as a *supercritical fluid.* (Skoog, 1985). Supercritical fluids are produced by heating a liquid above its critical temperature or compressing a gas above its critical pressure (see diagram). Under these conditions, the molar volume is the same whether the original form was a liquid or a gas. Supercritical fluids possess a very low surface tension, low viscosity, and high diffusivity, which allows fast mass transfer when compared with liquid solvents. Solvent strength can be controlled by varying the fluid pressure or the temperature or both.



Phase diagram of a pure substance showing the suprecritical state (above its critical temperature (and pressure), a substance exists as a supercritical fluid).

Supercritical fluid extraction (SFE) has the potential for providing rapid and quantitative extraction of organic pollutants from water, soil and air samples. SFE has been shown to yield quantitative extraction and recovery of organic pollutants from a variety of matrices, including soils, ait particulates and sorbent resins in 10 to 30 minutes, while yielding a concentrated sample immediately ready for further analysis.

Carbon dioxide has a critical temperature of 31°C and a critical pressure of 72.9 atm, conditions which are very well applicable in practice (Zeilemaker, 1995). Carbon dioxide is non-toxic, not flammable or explosive, chemically relatively inactive, and available in high purity at relatively low cost.
Polarity of supercritical carbon dioxide is comparable to liquid pentane, and thus it acts as a non-polar solvent, which is the main drawback of supercritical CO_2 when considering extraction of polar analytes.

An SFE experiment can be divided into three sequential steps: initial partitioning of the analyte from the matrix active sites into the supercritical fluid, elution of the analyte from the extraction cell, and collection of the analyte in the SFE trapping system, normally at ambient pressure (Bøwadt, 1995). Collection depends heavily on the restrictor system and trapping systems used.

SFE can be performed in either a dynamic or a static mode. For dynamic SFE, supercritical fluid is constantly flowing through the cell, and a flow restrictor is used to maintain pressure in the extraction vessel and allow the supercritical fluid to depressurize into the collection device. Static SFE is performed by pressurizing the cell and extracting the sample with no outflow of the supercritical fluid. After a set period of time, a valve is opened to allow the analytes to be swept into the collection device.

Extraction rates that are controlled primarily by the solubility/elution process show direct correlation with SFE flow rates (e.g., doubling the flow rate doubles the extraction rate), extraction rates that are controlled primarily by the kinetics of the initial desorption step show little or no change with different SFE flow rates.

ANNEX E ANALYTICAL METHODS

Narcotic mixture

Water samples were extracted with n-hexane with an internal standard. The extraction time was 5 min. Quantitative gas chromatographic analyses were performed with Carlo Erba Strumentazione, series HRGC 5300 GC, with a splitter SL 516, an autosampler AS800 and a J&W DB-5 analytical column (no. 9711827, 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.
GC conditions were: injection port 250°C, column temperature programmed from 45° to 70°C at a rate of 20°C/min., 1 min at 70°C, from 70°C at a rate of 5°C/min to 170°C and at a rate of 20°C/min to 270°C/min. Detector temperature: 280°C. Helium gas flow rate 2.0 mL/min.
For detection limits and further details the reader is referred to De Groot et al. 1996.

Pesticide mixture

Bentazone and Diuron were analysed by RPLC-UV according to SOP LOC/258/00.
Dichobenil, lindane, chlorfenvinphos, fenchlorphos and methyl-parathion were extracted twice or three times from water using dichloromethane.
After combining the aliquots, the dichloromethane was dried using sodium sulphate and dichloromethane was removed by Kuderna-Danish distillation. The residue was dissolved in ethyl acetate.
The compounds were analysed by means of GC-AED, GC-ECD or GC-FPD, according to SOP LOC/123/00. The GC was equipped with an automatic injector, FP-detector and an integrator. Column: fused silica, stationary phase DB 1301, 25 m, ID 0.32 mm, 1.0 µm film. GC conditions were: splitless injection, 4.0 µL; injection port 190°C, column temperature programmed from 80° (2 min) to 140°C at a rate of 30°C/min., and from 140°C at a rate of 3°C/min to 280°C. Temperature kept on 280°C for 15 min. Detector temperature: 300°C. Helium gas flow rate 1.8 mL/min.