Concentration techniques for genotoxicity testing of effluents

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Report number E01/08

December 2001

Study commissioned by the National Institute for Inland Water Management and Waste Water (RIZA). Contract nr. 39801/EMP.

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Preface

The present study was part of a preparatory programme on the introduction of genotoxicity testing and other acute and chronic bioassays in the integrated framework for biological and chemical evaluation of wastewater effluents (WEER; whole effluent environmental risk procedure; Totaal-Effluentbeoordeling) in the Netherlands. Based on previously made choices for the application of the UmuC-test, the main objective of the present study was to evaluate suitable preconcentration techniques, to define proper selection criteria, and to develop a proposal for a cost-effective preconcentration technique.

The project was commisioned by the National Institute for Inland Water Management and Waste Water (RIZA) of Rijkswaterstaat (Contract nr. 39801/EMP) under supervision of Dr Gert-Jan de Maagd. The project executed in a 10 week period by Drs. Mohamed Adahchour and Dr René Vreuls of the Department of Analytical Chemistry and Applied Spectroscopy (ACAS), Faculty of Exact Sciences (FEW), and Dr Bert van Hattum of the Institute for Environmental Studies (IVM), Faculty of Earth and Life Sciences (FALW), who took care of the overall project management. The authors acknowledge the support of Serge Rotteveel Msc and Ineke Blankesteijn Bsc (both from RIZA), Dr Freek Ariese (ACAS) and Dr Juliette Legler (IVM) for their part in the review of the project and usefull contributions.

Executive summary

In the present study, preconcentration methods were evaluated for the extraction and concentration of genotoxic compounds from effluents and surface water. The project was commissioned within the framework of the development of a whole effluent environmental risks procedure (WEER) by the National Institute for Inland Water Management and Waste Water Treatment (RIZA). Based on previously made choices for the application of the UmuC-test, the main objective of the present study was to evaluate suitable preconcentration techniques, to define proper selection criteria, and to develop a proposal for a cost-effective preconcentration technique.

To date, over 500 different organic and inorganic compounds have been tested for genotoxicity (mutagenicity, DNA damage, and carcinogenicity) with a large variety (appr. 200), of test systems, ranging form prokaryotic tests to mammalian species. Based on a selection of 156 compounds, responsive in the UmuC-test, physico-chemical properties, such as the *n*-octanol/water partitioning coefficient (K_{ow}) and the Henry Law Constant (H in $P.m^3/Mol$), were evaluated and classified. These parameters are considered as key factors describing the fate during sample-handling procedures and were important criteria for the selection of potentially suitable methods.

Measured values were derived from the Environmental Fate Data Base (EFDB, Syracuse Comp., USA). Missing values were estimated from structural parameters with the KOWWIN (fragment method) and HENRYWIN (bond contribution) modules in the EPIWIN suite from US-EPA. Genotoxic compound differ largely in hydrophobicity. Approximately 22 % was classified as hydrophilic (Log K_{ow} <0), 16% as polar (Log K_{ow} 0-1), 36% as medium-polar (Log K_{ow} 1-3) and 26% as hydrophobic (Log K_{ow} >3). The majority of the compounds (56%) was classified as relatively non-volatile form water (H < 10^{-3} $Pa.m^3/Mol$), approximately 29% as intermediate (10^{-3} < H < 1, and 14% as prone to volatile losses during sample-handling (H > 1), with 7% above H = 10.

An overview is presented of preconcentration techniques together with a more in-depth treatment of currently available SPE/SSPE techniques (solid phase extraction/ sequential solid phase extractions), some of the critical performance characteristics, such as breakthrough volume, and a provisional classification of compounds as used in this study. Basic properties of a selection of 22 SPE/SSPE procedures, derived from over 80 recent publications, are presented in the form of detailed factsheets in the Annex section. Especially the breakthrough volume appeared to be a critical factor. It was shown that most commonly used hydrophobic SPE materials exhibit breakthrough losses in the LogK_{ow} interval of 0-3, which coincides with LogK_{ow} of more than 50% of the UmuC-responsive compounds.

Based on the properties of responsive compounds and constraints from the UmuC-testing protocol a selection of 5 SPE/SSPE for surface water trace-enrichment methods was evaluated in more detail: C-18, Oasis HLB, Lichlolut EN, Isolut ENV+ and GCB based methods. For the four different hydrophobicity intervals the performance of the sorbents were evaluated with respect to: breakthrough volume, recovery, enrichment factor, and ease of drying.

A combined SSPE method , including C-18 followed by GCB was proposed as the most comprehensive method, expected to cover a wide range ($LogK_{ow}$:-2 to 6). Expected losses are likely to occur in the hydrophilic range (Log K_{ow}<-2; matching with 10% of the UmuC-responsive compounds) and approximately 5% (of the UmuC-responsive compounds) in the super-hydrophobic range (Log K_{ow} >6). Further losses due to evaporation during sample-handling may be present for approximately 14% (n=22) of the compounds, if a conservative cut-off of H=0 is chosen. A conservative estimate shows that approximately 70% of the UmuC-responsive compounds is expected to be extracted quantitatively with the proposed method. If the volatile fraction is of importance, than the additional application of purge-and-trap concentration techniques prior to genotoxicity testing is recommended.

The proposed C18/GCB SSPE procedure can be easily adapted to yield separate fractions (with different hydrophobicity class intervals) for further toxicity identity evaluation (TIE) studies. Several of the discussed sorbent materials can also be incorporated in a HPLC setting, for on-line separation in combination with automated fraction-collecting systems, dosing to e.g. microwell-plates which allow high-throughput testing with the UmuC-protocol.

With the current state-of-the-art in SPE/SSPE techniques it is not possible to design a single generic method, suitable for routine (waste)water screening, which is fully comprehensive. If compounds with extremely high or low hydrophobiciy are of importance, or the attention is dedicated to higly volatile compounds, special dedicated methods need to be applied. A comprehensive review of the literature revealed that three parameters are the most critical for the recovery in the final extract: volatility, breakthrough and desorption characteristics.

The category of inorganic compounds was not included in this study. Although in theory adding specific ion-exchange resins in sequence with the other SSPE may result in the trapping of metallic compounds of interest (e.g. selenium, chromium compounds), it is not possible to isolate the redox-state and chemical species, as applied in the original genotoxicity tests with dosages by far exceeding normal levels in effluents and surface water. We recommend not to include inorganics in the generic screening approach. In cases where genotoxicity of inorganics may be of importance (e.g. effluents of specific industrial sectors), dedicated studies are considered as the most cost-effective approach.

1. Introduction

The present study was commissioned within the framework of the development of a whole effluent environmental risk procedure (WEER) by the National Institute for Inland Water Management and Waste Water Treatment (RIZA). This integrated approach will combine ecotoxicological tests (acute- and chronic aquatic toxicity, bioaccumulation, genotoxicity and other *in-vitro* bioassays) in combination with traditional chemical approaches and dedicated toxicity identity evaluation (TIE) studies, to explain observed bioassay responses (Tonkes et al., 1998).

To date, over 500 different organic and inorganic compounds have been tested for genotoxicity (mutagenicity, DNA damage, carcinogenicity) with a large variety (appr. 200; de Maagd and Tonkes, 2000), of test systems, ranging form prokaryotic (e.g. bacterial) tests to mammalian species. The databases hosted by IARC (monographs with classification; www.iarc.lyon.fr) and the US-EPA/IARC (GAP database; www.epa.gov/gapdb) provide comprehensive and continuously updated information. Many of the genotoxic organic compounds are emitted from industrial and non-point sources and may end up in aquatic environments, depending on persistence, volatily and affinity to sediments and biota. Usually concentrations in surface waters and - to a lesser extent- effluents are below the no-effect ranges of genotoxicity tests, and some form of concentration may be required to elicit a response. A wealth of different preconcentration techniques has been applied for aquatic genotoxicity testing, and since long the need for validation and standardisation of sample-prep has been advocated (Stahl, 1991).

This study, addresses the selection of suitable concentration techniques for surface and wastewater, and covering a wide range of matrix and compound properties. From previous studies it is known that few methods exist that extract and concentrate all relevant compounds to an equal extent (Stahl, 1991; de Maagd, 2001) and that volatile or highly polar genotoxic compounds may be underrepresented in concentrated extracts (Vargas et al., 1995). Some authors have reported on variation in genotoxic response of wastewater extracted and concentrated with different methods (Gauthier, 1993). Filipic and Toman (1996) stated that the genotoxic response of concentrated samples cannot be extrapolated directly to the response of the original sample.

In previous studies, various genotoxicity tests were evaluated for their suitability for screening of wastewater and surface water (Klamer et al., 1997; De Maagd et al., 1999; De Maagd & Tonkes, 2000) and the UmuC-test (Reiferscheid et al., 1996; Hamer et al., 2000) was assigned as one of the candidate prokaryotic tests for primary DNA-damage in the WEER programme. Before implementation various issues need to be addressed, ranging from optimisation and standardisation of sample-handling procedures to protocol development, validation and intercalibration studies (de Maagd et al., 1999).

The main goal for the present satellite-project was to review existing preconcentration methods for (waste)water and to evaluate and select the most promising methods for application in the UmuC-test procedure. As the currently known set of genotoxic compounds exhibit order of magnitude variations in their physico-chemical properties, special attention was given to the comprehensiveness for the full range of compounds.

The following objectives were defined:

- to review the state-of-the-art of currently known concentration techniques suitable for combination with aquatic genotoxicity testing;
- to inventorise and evaluate some key physico-chemical properties of responsive compounds: the *n*-octanol/water partitioning coefficient (K_{ow}) and the Henry's Law Constant (H);
- to formulate and apply selection criteria to identify the most promising methods;
- to discuss gaps in knowledge and relevant developments in the field of sample-prep in the near future.

In Chapter-2 an overview of preconcentration techniques is presented, together with a more in-depth treatment of currently available SPE/SSPE techniques (solid phase extraction/ sequential solid phase extractions), some of the critical performance characteristics, such as breakthrough volume, and a provisional classification of compounds as used in this study. Basic properties of a selection of more than 30 SPE/SSPE procedures, derived from over 80 recent publications, is presented in the form of detailed factsheets in Annex-1. Estimated and experimentally determined properties (K_{ow} and H) of UmuC-responsive compounds are presented in Annex 2-4 and are evaluated and analysed in Chapter-3. Based on the properties of responsive compounds and constraints from the UmuC-testing protocol a selection of 5 SPE/SSPE methods was evaluated in more detail. The development and application of selection criteria, resulting in a proposed combination of SPE methods is presented in Chapter-4. After a brief discussion of gaps in knowledge and future development in de field (Chapter-5), conclusions and recommendations were summarized in Chapter-6.

2. Concentration techniques

2.1 Introduction

Many organic contaminants are present in surface waters in trace amounts at the µg/L level and often below. As a result, very low detection limits are required for direct monitoring. To date, several hundred organic contaminants have been classified as genotoxic (Reifferscheid et al., 1996). Because of the low concentrations, detecting them without some sort of concentration step would be nearly impossible. For that purpose various concentration techniques have been used, including lyophilization, reverse osmosis, liquid-liquid extraction, solid-phase extraction, etc. In general, sample preparation is the most tedious and time-consuming step. It is also a potential contributor to the imprecision and inaccuracy of the overall analysis, especially when the sample (extract) is used for toxicity testing (Stahl et al., 1991; de Maagd et al., 1999). Therefore, the choice of the method(s) for the concentration and isolation of the various types and classes of contaminants (toxicants) from water samples is complicated and depends on the analyte characteristics (polarity, ionic character, stability) and other parameters related to the applied method.

In this section of the report, a general discussion and classification will be presented of the concentration techniques that were most widely used in the last decade, which will help to define the selection criteria presented in section 4.

Articles were selected from the following three main databases: chemical abstracts, web of science and science direct. The search strategy was based on the following keywords: concentration techniques, solid-phase extraction, liquid-liquid extraction, water samples, polar compounds, isolation techniques, toxicity test, toxic compounds, genotoxic compounds, polar organic contaminants, effluent, waste water, ground water, tap water, UmuC-test. Beside these keywords a search was conducted on specific groups of compounds, according to the selected list of genotoxic analytes presented in section 3.

2.2 General survey and methods classification

As mentioned above, there are still many problems encountered in the isolation and preconcentration of organic compounds in water samples. Concentrating the sample may produce artefacts, sometimes leading to both false positive/negative results. Different concentration techniques have been used in the past to overcome such problems:

Lyophilization. Lyophilization, the removal of water by vacuum sublimation of ice, is a method for concentration in-volatile organic compounds in aqueous solution and for preserving biological samples. The essential steps are that the sample is first frozen and then placed under high vacuum. The heat absorbed by the sample from its surroundings causes the ice to sublime, which is then re-condensed on a large surface condenser held at cryogenic temperatures. This technique, also known as freeze frying, has several drawbacks. First, salts, bacteria, and other materials in the sample are not removed and may interfere with the genotoxicity test. Second, only small samples can be processed

(generally < 100 ml) which reduces the concentration factor achievable. The advantage is that an aqueous solution is obtained, which is fully compatible with the UmuC-test.

Reverse osmosis and evaporation. Reverse osmosis is a separation process in which (large) molecules are separated from solution by filtration through membranes. The operating pressure must exceed the natural osmotic pressure for the system resulting in the movement of solvent; usually water, from the solution of high analyte concentration to that of low analyte concentration.

Next to the drawbacks mentioned for lyophilization, reverse osmosis and evaporation can be inefficient and time consuming. These approaches have the potential to markedly alter the composition of a sample through sorption, leaching, evaporation, or degradation of the contaminants.

Freeze concentration. Unlike lyophilization, freeze concentration has been used for the concentration of aqueous solutions of organic volatiles and substances that are heat labile. For successful results, the contact layers between the liquid and solid phases should be continuously disturbed by stirring or shaking and part of the solution should remain unfrozen at the end of the concentrating procedure.

The most important weakness of the freeze concentration method lies in its completeness. Increasing the total dissolved solids concentration of highly mineralised samples could potentially elicit toxic effects from ion imbalance. Conductivities greater than 2,000 µS/cm may be high enough to adversely affect freshwater species.

Liquid-liquid extraction (LLE). LLE has remained the preferred technique for the preparation of liquid samples for several years, especially in the environmental field, and is still widely used in standard and official methods. Its process is based on distribution of analytes between two immiscible solvents in which analytes have different solubilities. The efficiency of an extracting solvent depends on the affinity of a solute for the extracting solvent, the phase ratio and the number of extractions.

LLE with traditional organic solvents is more time-consuming than the other methods discussed, but provides qualitative information about the classes of organic compounds causing the genotoxic activity, i.e., acid, neutral, or basic compounds. However, the introduction of survey lists (see Chapter 3) containing (very) polar analytes such as some degradation products of organic micro-pollutants has also pointed out the need for alternative methods to LLE. Many polar analytes are partly soluble in water and cannot be extracted with good recoveries, whatever the organic solvent selected. Other disadvantages of this technique, *e.g.* matrix interferences, emulsion formation, the use of large volumes of hazardous solvents, have troubled the analyst.

Solid-phase extraction. SPE is an extraction and enrichment technique and its process, to a first approximation, can be considered as a simple liquid chromatographic process. The sorbent is the stationary phase and the mobile phase is the water constituting the aqueous sample during the extraction step and the organic solvent during the elution step. Compounds that do not elute with the water are trapped on the sorbent during the percolation step. High enrichment factors are obtained when analytes are strongly retained by the sorbent in the presence of water, allowing the percolation of a large volume of sample and when there is a low retention when eluting by organic solvents.

Solid-phase extraction (SPE) has been developed as an alternative to LLE (and other extraction techniques) and can (partially) overcome its drawbacks. Motivation of this choice is presented in some detail in the next section.

2.3 Motivation for the selection of SPE

The increasing popularity of SPE is resulting not only from its obvious advantages over LLE (e.g., minimised consumption of organic solvents, no emulsion formation, reduced contact for the analyst with potentially toxic substances), but also from the gradual refinements of this procedure, which minimise its original drawbacks. A wide choice of newly developed material, belonging mostly to one of the three major groups of sorbents (i.e., bonded silicas, polymers and carbon materials), makes SPE a suitable tool to scope with an increasing variability of modern organic contaminants. The theory and method development of SPE for water analysis were recently reviewed (Poole et al., 2000; Liska 2000; Hennion 1999; Hennion 2000; Huck et al., 2000; Leon-Gonzalez et al., 2000 and Pichonm, 2000). The applicability of SPE for a specific group of analytes has also been reviewed, including phenols (Rodriguez et al., 2000 and Bruzzoniti et al., 2000), acidic herbicides (Wells et al., 2000) and pesticides, triazines and degradation products (Sabik et al., 2000).

Different laboratories have studied comparison of SPE to LLE extensively. Di Corcia *et al.* (1993) compared the recoveries of 20 polar pesticides obtained from 2 L of water using a 1-g graphitized carbon black (GCB) cartridge to those obtained with using a 1-g C_{18} silica cartridge or by using LLE with three separate 120-ml portions of methylene chloride. SPE (GCB) was superior to LLE, with recoveries of 75-102% and 13-89% respectively. The recoveries obtained with 1-g C_{18} were much lower (3-55%, except for isocarbamid, hexazinon and metoxuron with recoveries higher than 80%) because breakthrough (see paragraph 2.5 for more details) occurred for most of the pesticides with a sample volume far lower than 2 L. Tolgyessy et al. (1999) investigated the efficiency of pre-concentration of selected organic compounds from aqueous solutions on various SPE materials including bonded silica, polymers and carbon materials. Simultaneously, the potential of newly emerging SPE procedures was compared to the results of traditional LLE methods using various organic solvents. A group of 19 test analytes was selected so as to represent different classes of organic compounds, which may occur in waters. The results showed that most of the tested materials were suitable for sufficient preconcentration of a substantial part of the tested analytes. However, specific differences in the recovery of one or more analytes were found for almost each sorbent, even in the case when the materials had a similar composition. This behaviour clearly indicates the need of a thorough testing of the capabilities of any SPE material intended for the identification of (un)known organic micropollutants of a wide polarity range in waters.

2.4 Sorbents for SPE

The key problem when applying SPE remains the method development and the primary decision for the analysts is the choice of the sorbent that is able to solve their trace-analysis problem. Before selecting a sorbent for SPE, it is necessary to take into account some physico-chemical aspects (Chapter 3), such as the functional groups of the analytes, the nature of the bonded phase, the energetics of the interactions, the secondary in-

n-
teractions between the sorbent and the components of the sample matrix, and the interactions between the analytes and the sample matrix. Sorbent-analyte interactions fall into three categories: non-polar, polar and ionic.

To date, typical SPE materials are modified silicas with C_8, C_{18}, CN and other groups, carbon blacks and styrene-divinylbenzene copolymers (PS-DVB). The most problematic compounds are the polar ones, for which breakthrough volumes are low with most of the sorbents mentioned. This has encouraged investigators to evaluate alternative sorbent materials, such as chemically modified polymeric resins, highly cross-linked polymers and others.

Modified silica. Since the retention mechanism is primarily governed by hydrophobic interactions between the analyte and the carbon moieties of the alkyl chain, a relation has been observed between the retention factor of the analyte and its *n*-octanol/water partition coefficient (K_{ow}). It was demonstrated that, with an amount of sorbent of 500 mg, a recovery in the range 90-100% for a percolated volume of 500 ml was found for compounds with a Log $K_w > 3$ (Hennion et al., 1998); K_w is the retention factor of the solut eluted by water (see Section 2.5). This guidance value is the basis of the choice of the sorbent. No special problems occur during the extraction of moderately or non-polar analytes. However, these sorbents are inadequate for solving the problems involved in isolating polar contaminants from large water volumes (Di Corcia et al., 1993). Apart from that, these sorbents suffer from chemical limitations, such as residual surface silanol groups, a narrow pH stability range and poor selectivity.

Graphitized carbon black (GCB). This material is characterised by a highly homogeneous and ordered structure and by a specific surface area of ca. 120 m^2/g. It has been shown that organic compounds are adsorbed on a GCB surface more strongly than on a C_{18} surface when analysing polar compounds in water (Di Corcia et al., 1993, Hennion et al., 1998). The retention mechanism differs from the one observed on C_{18} silica or PS-DVB polymers due to its crystalline structure made of large sheets held together by weak Van der Waals forces (Hennion et al., 1995). Analytes are retained on GCB by both hydrophilic- and electronic-type interactions, so that non-polar but also very polar and water-soluble analytes have been shown to be strongly retained in aqueous mobile phases (Guenu et al., 1996a and b). A new GCB sorbent, Carbograph 4, was used to determine phenolic compounds in water and they yielded better recoveries for the most polar analytes than Carbograph 1 (Pocurull et al., 1996 and Di Corcia et al., 1996).

A drawback of the GCB sorbents are that they result in excessive retention (some analytes can even be irreversibly adsorbed) but this can be overcome by performing the elution in the back flush mode (Di Corcia et al., 1996). Its mechanical property is also poor; which results in low-pressure resistance to be used in liquid chromatography. Porous immobilised graphitic carbon (PGC) is a new carbon –based sorbent in which the graphite is immobilized on a silica structure, and this is why PGC is more stable than GCB. The use of PGC was investigated for the trace enrichment of polar phenolic compounds and compared with polymeric sorbents. Except for 4-chloro-2-aminophenol, the breakthrough volumes and detection limits were worse with PGC than those obtained using the polymeric sorbents (Puig et al., 1996).

-ð interactions with unsaturated analytes. The efficiency of the polymeric sorbents depends on various physico-chemical parameters such as particle size, surface area, pore diameter, pore volume, degree of cross-linking and particle size distribution (Puig et al., 1996 and Hennion et al., 1998). Resins with high specific surface areas in the range 700 to 1200 m^2/g, are now available in disposable cartridges and its effectiveness has been demonstrated by Hennion et al. (1998).

In recent years, chemically modified resins containing different polar functional groups have been developed and used in the SPE of polar compounds from environmental waters. These modified resins have excellent hydrophilicity and they also give higher recoveries than their unmodified analogues (Masque et al., 1997). This has been attributed to an increase in surface polarity, which enables the aqueous sample to make better contact with the resins' surface. They have also been compared with other SPE materials such as PLRP-S and GCB for the preconcentration of pesticides and phenolic compounds, and they yielded higher breakthrough volumes for the most polar compounds than PLRP-S and GCB (Masque et al., 1997 and Masque et al., 1997). In the last few years, several new highly cross-linked polymers, such as Envi-Chrom P, LiChrolut EN, Styrosorb, Isolut ENV and HYSphere-1, have become available. These highly cross-linked PS-DVB sorbents are the sorbents to be selected for the extraction of very polar analytes when large sample volumes are required. Many examples can be found in the recent literature (Castillo et al., 2001; Steen et al., 2001; Lopez de Alda et al., 2001 and la Farre et al., 2001a-d).

One limitation of both reversed-phase silica and polymer sorbents is that they must be conditioned with a wetting solvent and remain wetted until sample application. One new patented sorbent has been recently introduced in the market. It is the so-called hydrophilic-liphophilic balanced sorbent (Oasis HLB from Waters), which is a copolymer of divinylbenzene and *N*-vinylpyrrolidone (Bobeldijk et al., 2001a and Bobeldijk et al., 2001b). It is presented as the "universal" extraction sorbent since it is capable of extracting acidic, basic and neutral compounds, whether polar or non-polar.

This new hydrophilic–lipophilic polymer is (more) suitable for the handling of biological samples like serum. There is an "universal procedure" which consists of applying 1-ml of sample, washing with 1 ml of 5% methanol before eluting with 1 ml of methanol. Since the sorbent combines a high specific surface area with some polar hydrogen groups, it is easy to explain why there is no breakthrough of analytes with 1 ml of samples whatever the organic analyte, being polar, neutral or ionised, even when 5% methanol is added for the washing step. The new polymer is designed to extract an extensive spectrum of analytes, i.e., lipophilic, hydrophilic, acidic, basic and neutral with a single cartridge with a simplified procedure since no conditioning is required. But the universal procedure (apply 1 ml of sample, wash and elute) is limited to the handling of biological samples since the sample volume that is recommended is 1 ml. More work should be

done to look at the limitation in the extraction of polar analytes from relatively high (water) sample volumes as it was done for other highly cross-linked PS-DVB.

To guarantee the success of the SPE process for a particular application, it is of high importance to understand the effect of the most critical parameters on analyte recovery during the SPE procedure. In chapter 4, a selection criterion is proposed according the described goal in the Introduction to select (an) optimal method(s).

2.5 Breakthrough volumes *versus* Log K_{ow}

The breakthrough volume is the most important parameter for determining the suitability of a sampling device for isolating the analytes of interest. It represents the maximum sample volume, which can be percolated with a theoretical 100% recovery. In the initial sampling phase a sample of fixed concentration, and usually at a constant velocity, enters the sampling device. The analytes are quantitatively retained during the initial sampling phase by the sorbent, up to the point that the sample volume exceeds the retention capacity of the sorbent.

A typical example of the relation between breakthrough volume and Log K_{ow} of a group of pesticides (van Loon et al., 1996; Liska et al., 1992; Brouwer et al., 1991; Hennion et al., 1993) is shown in Figure 2.1. From this figure, it is clear that compounds with Log $K_{ow} < 1$ have a breakthrough volume smaller than 10 ml. For compounds with Log $K_{ow} > 3$, the breakthrough volume is higher than 100 ml. In the 1–3 Log K_{ow} range, breakthrough volume increases with Log K_{ow}. To recover the (very) polar compounds, a polar phase is needed i.e., GCB or PS-DVB with high specific surface areas.

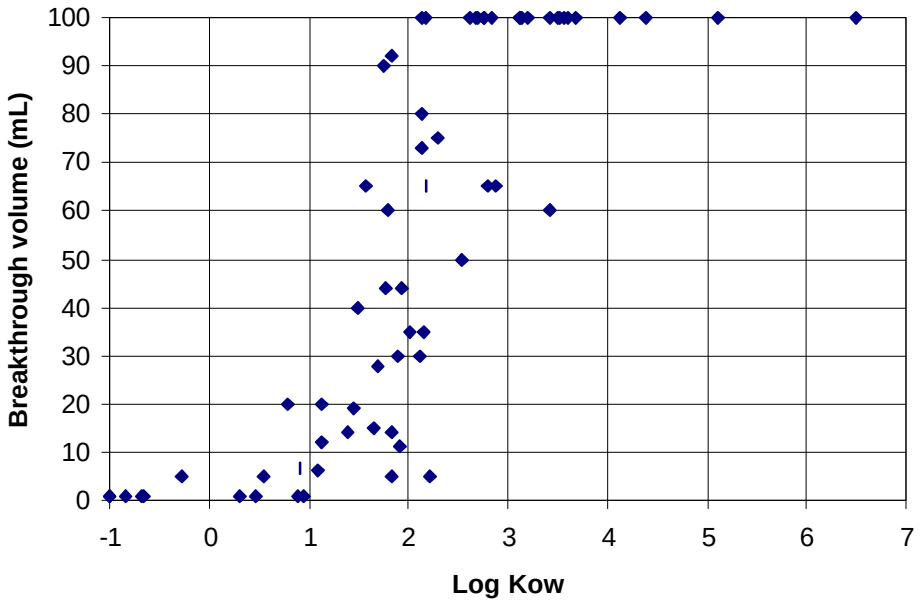


Figure 2.1 Relation between Log K_{ow} and breakthrough volume for 65 pesticides and other compounds on polymeric phases (PRLP-S and PRP), L 10 mm x ID 2 mm. Adapted from: Van Loon et al. (1996), Liska et al. (1992), Brouwer et al. (1991) and Hennion et al. (1993).

The breakthrough volume can be calculated or estimated by the following well known empirical equation:

$$V_b = (V_{geom} \cdot å)(1 - 2/N^{0.5})\ 10^{a\ LogK_{ow} - b}$$

where V_b is the breakthrough volume, V_{geom} is the geometric volume of the column or the cartridge, å is the porosity of the sorbent, N is the number of theoretical plates, K_{ow} is the *n*-octanol/water coefficient of the solute, and a and b are the linear regression coefficient of the relationship between $LogK_{ow}$ and $LogK_w$ (retention factor of the solute eluted by water). With average values of density and porosity of the sorbent used in cartridges, the void volume V_m ($V_m = V_{geom} \cdot å$) of the cartridge can be calculated. For example, V_m for C_{18} silica is estimated as 0.12±0.01 ml per 100 mg of sorbent. It is much more difficult to measure the efficiency of an SPE cartridge or that of an extraction disk, so that N has to be estimated.

The determination of breakthrough volumes, particularly by off-line methods, is time-consuming. It can be estimated from solute properties using models that require a minimal number of experimental measurements. The breakthrough curves can be easily been modeled according to the above equation, provided that values are known for porosity of the sorbent, number of plates and amount of sorbent (Hennion et al., 1998). These curves have been constructed with the hypothesis of an amount of 450 mg of sorbent, an average porosity of 0.70 and a number of plates of 20. The effect of the number of plates was shown to be negligible when modelling with the same conditions. The only strong effect is the amount of sorbent, but the relation between V_b and V_m is straightforward in the equation mentioned above.

This model has been validated by experimental measurements of recoveries for several analytes using extraction disk packed with C_{18} silica and PS-DVB sorbents (Hennion et al., 1998). A good agreement was obtained between experimental and modelled curves for oxamyl having Log Kw values of 1.7 and 2.8 on C_{18} silica and PS-DVB sorbent and desethylatrazine with Log Kw value of 3.5 on the PS-DVB sorbent. A great difference in sample volumes that can be percolated with a good recovery was observed when using a disk containing C_{18} silica or the PS-DVB copolymer. Taking a value of 90% for recovery, the corresponding sample volumes were, respectively, 30 and 300 ml for oxamyl on the two sorbents.

The *n*-octanol/water partition coefficient has become a recognised parameter to estimate compound hydrophobicity of general importance for modelling numerous environmental and biological properties associated with the use and disposal of organic compounds. Nakamura et al. (1996) proposed a general guide for the selection of sample processing conditions for the isolation and recovery of agricultural chemicals from water based on estimated Log K_{ow} values. A group of empirical rules were presented in the form of a decision tree and said to be useful for selecting reversed-phase sorbents and organic solvents to aid experimental trial-and-error approaches to method development. However, for very polar analytes, Log K_{ow} was shown to be of limited help for predicting the SPE recoveries, especially for very polar analytes with Log $K_{ow} < 1.5$ (Hennion et al., 1998). Since the retention mechanism is primarily governed by hydrophobic interactions between the analyte and the carbonaceous moieties of the alkyl chains grafted at the silica surface, a relation has been observed between the retention factors of the analytes and

their K_{ow}. Few data have been published with regards to polar analytes, especially for very polar analytes with Log K_{ow} below 1.5. For these compounds, a large difference is observed between Log K_{ow} and extrapolated Log K_w values. For very polar analytes, a more rapid method is certainly to have in the laboratory a 10- or 5-cm long C_{18} column, and to extrapolate log K_w from K measurement in a methanol–water mixture containing as high as possible water content. This is rapid and can be easily with autosampler and HPLC devices. Table 2.1 shows the extrapolated Log K_{ow} values from the relationships Log K–percentage of methanol of 14 different compounds (Log K_{ow} –1.2–2.4 range). Five different cartridges and disks were compared including, $C_{18,}$ PRP-1, PS-DVB (disk), high surface area/PS-DVB (HSA/PS-DVB) and PGC (carbon). It is clear that these HSA/PS-DVB (LiChrolut EN or Isolut NV+) are the sorbents to be selected for the extraction of (very) polar analytes when large sample volumes are required (Hennion et al., 1998; Pichon et al., 1998). The potential of PGC for extracting very polar compounds is shown in Table 2.1 for dealkylated and hydroxylated degradation products of atrazine down to cyanuric acid. Most of them have Log K_{ow} values lower than 0. The limitation of other sorbents is clearly shown for the very polar ammeline, ammelide and cyanuric acid with Log K_w values lower than 0.5 whereas they are higher than 2 with PGC. Using a 200-mg PGC cartridge, recoveries were above 90% with the handling of 250 ml of water sample for all the metabolites except the three more polar ones for which a 500-mg cartridge was required to obtained similar recoveries.

Table2.1. Comparison of Log K_w values obtained with C_{18} silicas, various PS-DVB copolymers and porous graphitic carbon (Hennion et al., 1998).

Compounds	Log K_{ow}	Log K_w				
		C_{18}	PRP-1	PS-DVB (disk)	HAS/PS-DVB	PGC
Cyanuric acid	-0.2	< 0.5	< 0.5	nd	< 0.5	2.6±0.1
Ammeline	-1.2	< 0.5	< 0.5	nd	< 0.5	2.4±0.2
Ammelide	-0.7	< 0.5	< 0.5	nd	< 0.5	2.5±0.2
Hydroxy-DIA	-0.1	1.0±		nd	1.8±0.1	3.0±0.2
Hydroxy-DEA	0.2	1.5±		nd	2.3±0.2	2.8±0.2
DEDIA	0	1.3±		nd	nd	2.8±0.1
Deisopropylatrazine (DIA)	1.2	2.3±		3.2±	4.4±0.3	> 3.5
Deethylatrazine (DEA)	1.4	2.7±		3.5±	4.8±0.3	3.2±0.2
Simazine	2.3	3.4±		4.1±	5.9±0.3	> 4
2-Chlorophenol	2.4	2.9±		3.6±		> 4
Oxamyl	-0.47	1.7±		2.8±	4.1±0.3	nd
Aldicarb	1.4	2.5±		4.0±	5.3±0.3	nd
Carbendazim	1.5			nd	5.7±0.3	> 4
Chloridazon	1.6	2.3±		3.8±		> 4

Log K_w values extrapolated from the relationships Log K–percentage of methanol.
Cyanuric acid: 2,4,6-trihydroxy-1,3,5-triazine; ammeline: 2,4-diamino-1,3,5-triazine; ammelide: 2-amino-4,6-dihydroxy-1,3,5-triazine.

2.6 Compound classification

The compounds of interest have been divided in groups based on their *n*-octanol/water partitioning and volatility from water. Compounds, which are preferentially in the *n*-

octanol phase (> 99.9 % in a 1/1 v/v *n*-octanol/water system) were designed as hydrophobic (Log K_{ow} > 3) and in the water phase (> 50%) as hydrophilic (log K_{ow} <0). Compounds in the range Log K_{ow} 1–3 were designated as medium polar and those in the range 0–1 as polar. For the volatility several parameters are available, i.e. vapour pressure (Pv) describing the volatility of the pure compound, the boiling point describing the potential to separate compounds in GC chromatography, and the Henry Law Constant (H in $Pa.m^3/Mol$) describing the partitioning of a compound between air and water, and therefore taken as the best indicator for the evaluation of volatility losses from water.

For the classification we used the value proposed by Thomas (1990) for involatiles from water (Log H < -3), arbitrarily assigned categories of low (Log H –3 to –2) and intermediate volatility (Log H –2 to 1), and for the category of volatiles (Log H > 1) the properties of one of the least volatile compounds that can be analysed with purge and trap techniques, i.e. naphthalene (Log H = 1.6). This scheme is similar to the classification proposed by EPA (2000): non volatile (Log H < -3), slightly volatile (Log H –3 to –1), moderately volatile (Log H –1 to 1), volatile (Log H 1 to 3) and very volatile (Log H > 3). This classification has been used in the rest of the text and summarised in the following table.

Table 2.2 Operational classification of compounds for this study.

Compounds	Operational classification
Hydrophobic	Log K_{ow} range: > 3
Medium polar	Log K_{ow} range: 1-3
Polar	Log K_{ow} range: 0-1
Hydrophilic	Log K_{ow} range: < 0
Volatile	Log H* range: > 1
Intermediate	Log H range: -2 to 1
Low-volatility	Log H range: -3 to -2
Involatile from water	Log H range: < -3

* H in $Pa.m^3/mol$.

3. Physico-chemical properties of responsive compounds

3.1 Introduction

For a proper assessment of the suitability of concentration methods and sample-handling procedures it is necessary to evaluate a number of key properties of the target compounds. For this study, two main properties were selected: the *n*-octanol/water partitioning coefficient (K_{ow}), which is an important parameter for the prediction of the affinity to different sorbent materials, and the Henry Law coefficient (H), describing the partitioning between air and water and providing information on potential evaporative losses during sample handling.

The review of Reifferscheid et al. (1996), which contains a list over 450 compounds which have been tested with the UmuC-test, was taken as a starting point. The list of positive compounds (n=171) in the UmuC- test is included in Annex-2. The list comprises names and CAS-nrs of compounds which exhibited response without or after metabolic activation (S9; n= 32).

Other sources for databases of genotoxic properties of compounds, such as IARC (http://www.iarc.fr) and the EPA/IARC-GAP database (http://www.epa.gov/gapdb) containing comprehensive information on approximately 500 chemicals, were not used as it was difficult to isolate the UmuC-responsive compounds. For further exercises on compounds responsive in other test systems especially the IPA/IARC-GAP database seems very useful.

Based on information provided by RIZA, 37 compounds of the list in Annex-2, have been identified previously in XAD-extracts from River Rhine or Meuse water during monitoring studies conducted by RIZA (pers. comm. Dr. J. Staeb).

Measured values of K_{ow} and H were derived from the Environmental Fate database of Syracuse (SRC, 1998), which are also included in the EPIWIN software, available from US-EPA (Meylan and Howard, 1999a), and used for the prediction of K_{ow} and H for compounds for which no measured data were available. In the following sections a brief description is given of the estimation-software used. For more detailed descriptions of the methodological aspects and the comparison with other estimation algorithms we refer to Boethling and Mackay (eds., 2000), Meylan and Howard (1999a), and the documentation available from the EPIWIN website:
www.epa.gov/opptintr/exposure/docs/episuite.htm

3.2 Estimation of K_{ow}

The KOWWIN version 1.6 software (Meylan and Howard (1999c) was used to predict *n*-octanol/water partitioning coefficients (log K_{ow}) of organic compounds. The estimation procedure is based on the Atom/Fragment Contribution (AFC) method described by Meylan and Howard (1995) and differs from the fragment constant-based ClogP programme (Hansch and Leo,1979; Leo 1993; Leo 1995). Both methods provide relatively good predictions of K_{ow} and are generally recommended (Leo, 2000).

The KOWWIN program requires input as a CASnr (or other compound database formats, such as MDL Mol files). For unknown compounds, not included in the database, structures are entered as SMILES notation (Simplified Molecular Input Line Entry System). The output of the calculated Log K_{ow} consists of the contributions of different structural fragments and correction factors. A comparison is made with a database of over 12000 experimentally determined Log K_{ow} values.

Currently, KOWWIN has been tested on a validation dataset of 10,338 compounds. Regression statistics for predicted versus experimentally Log K_{ow} values were: r2 = 0.94; sd = 0.47; me = 0.35 (Meyan and Howard, 1999c).

Casnrs from the list of responsive compounds (n=159 excluding inorganic compounds) were entered in the KOWWIN programme. For approximately 89 % of the compounds matching CASnrs were found directly, and after correction this increased to 94%. For remaining compounds (n=9; 6%) Smiles notations needed to be entered. Smiles notations were either derived from Chemfinder (CambridgeSoft Corp., Massachussets MA, USA; www.chemfinder.com) or created manually. For 100 (62%) of the compounds experimentally derived values were available and used in the further analysis.

3.3 Estimation of Henry's Law Constants

The volatility of compounds usually are described with: the boiling point, the vapour pressure (V_p in Pa) and the Henry Law Constant (H), which can be regarded as e measure for the air/water partitioning of a compound, and is defined as a function of the vapour pressure and the aqueous solubility S (Mol/m^3):

$$H = V_p / S \qquad [\text{Pa.m}^3/\text{Mol}]$$

The vapour pressure usually is regarded as a measure for the partitioning of the pure compound and the atmosphere; the boiling point provides an indication of the GC-amenity of a compound, and H can be regarded as a good indicator for the ability to extract a compound (H > 40) with purge-and-trap techniques (Van Loon, 1996)

The software program HENRYWIN version 3 estimates the Henry's Law Constant (H) based on the bond and the group contribution method (Hine and Mokerjee 1975; Meylan and Howard 1991 and 1999b; Mackay et al., 2000). The group method estimate is generally preferred (Howard, 1999b) when all fragment values are available (which was not the case for many of the compounds in this study). The bond contribution method is able to estimate many more types of structures and was designated as more accurate than the group method in an evaluation by Altschuh et al. (1999). In the review of Mackay et al. (2000) the bond contribution method was one of the two recommended methods.

The programme includes an experimental Henry's law constant database of 1650 compounds. The experimental database of 1650 compounds is comprised of values that were either (1) measured directly or (2) derived from reliable vapor pressure and water solubility data (584 were measured directly). Values measured directly are indicated in the output together with the reference source of the experimental data. Values derived from vapor pressure and water solubility are referred to a SRC and list both the vapor pressure and water solubility and the derived Henry's law constant. The reference source of the vapor pressure and water solubility are not reported; they are taken from SRC's

PHYSPROP Database. Reliable vapor pressure and water solubility data have been shown to derive very accurate Henry's law constants (Meylan and Howard, 1991).

Meylan and Howard (1999b) report that major estimation errors may occur when the bond estimation is less than 1 x 10^{-8} atm.m^3/Mol (appr. 10^{-4} Pa.m^3/Mol). Compounds with a H < 3.10^{-3} Pa.m^3/Mol are considered as non-volatile from water (Thomas, 1990).

Based on information provided in the manual (Meylan and Howard, 1999b) the statistics of the regression of predicted versus experimental values were: r^2 = 0.85; s.d. = 0.87; me = 0.51 (n=1293; compounds with H < 10^{-4} Pa.m^3/Mol not included).

The HENYWIN 3.0 programme requires a similar input format as the KOWWIN programme. The output consists of experimentally-derived values for H at 25°C (in atm.m^3/Mol) together with results from the bond and (if available) the group contribution method. The options for different temperature corrections were not used.

Group contribution derived predictions were available for 73 compounds (46%). Based on the recommendations in the reviews of Mackay et al. (2000) and for reasons of consistency only bond-contribution estimations were used for compounds for which no measured values were available. For 39 (24%) of the compounds experimentally derived values were available and used in the further analysis.

3.4 Distribution of hydrophobicity

Estimated K_{ow} values and (if available) experimentally derived values (including source references) are listed in Annex-3. In Figure 3.1 and Table 3.1 frequency distributions are presented of the measured and predicted values for Log K_{ow} of the selected 159 UmuC-responsive compounds.

Table 3.1 Frequency distribution of Log K_{ow} of UmuC-responsive compounds.

LogK_{ow} class	All compounds			S-9 only		
	Frequency	%	Cumulative %	Frequency	%	Cumulative %
-4	1	.6%	.6%	1	3%	3%
-4 to -3	0	.0%	.6%	0	0%	3%
-2	5	3.1%	3.8%	0	0%	3%
-1	6	3.8%	7.5%	0	0%	3%
-1 to 0	23	14.5%	22.0%	2	6%	10%
0 to 1	25	15.7%	37.7%	3	10%	19%
1 to 2	35	22.0%	59.7%	10	32%	52%
2 to 3	23	14.5%	74.2%	4	13%	65%
4	21	13.2%	87.4%	5	16%	81%
5	11	6.9%	94.3%	1	3%	84%
6	4	2.5%	96.9%	3	10%	94%
7	3	1.9%	98.7%	1	3%	97%
7 to 8	2	1.3%	100.0%	1	3%	100%
>8	0		100.0%	0		100%
Total	159			39		

S9-only: selection of compounds responsive after metabolic activation (S9).

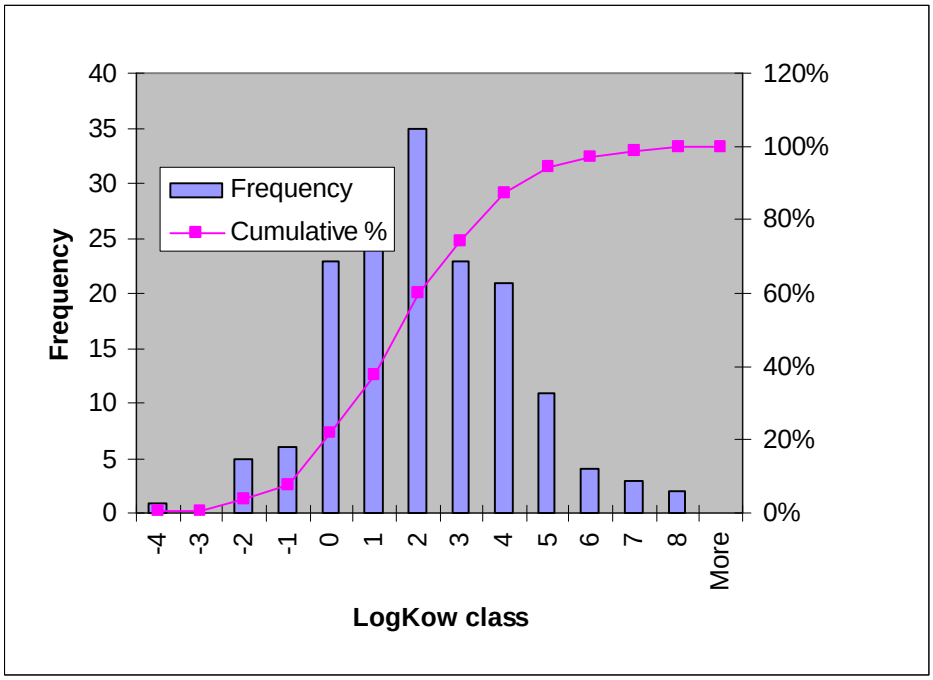


Figure 3.1 Frequency distribution of predicted (n=59) and experimentally derived (n=100) Log-K_{ow} values for UmuC-positive compounds.

Table 3.2 Frequency distribution over classes of Log K_{ow}.

LogK_{ow} class	All	comp.	S-9	only	RIZA	only
	Frequency	%	Frequency	%	Frequency	%
<0	35	22%	3	10%	3	8%
0-1	25	16%	3	10%	6	17%
1 to 3	58	36%	14	45%	17	47%
3 to 6	36	23%	9	29%	8	22%
>6	5	3%	2	6%	2	6%
Total	159		31		36	

Its is apparent that the UmuC-responsive compounds differ largely in hydrophobicity (Log K_{ow} –4< to >8). It should be noted that for both the hydrophilic compounds (Log K_{ow} <0) and the super-hydrophobics (Log K_{ow} > 8) the predictions tend to have a high uncertainty.

Observations for the different hydrophobicity classes are given in Table 3.2 Several conclusions can be drawn from the frequency distributions:

- UmuC-responsive compounds show large differences in their *n*-octanol/water partitioning coefficient. Approximately 52% of the responsive compounds falls within the class-ranges of polar (16%) to semi-polar (36%) substances. Hydrophobic compounds constitute 26% (of which 3% with Log K_{ow} > 6) and hydrophilic compounds approximately 22%.

- The group of compounds that is responsive after S9-metabolic activation (n=31) seems to have a slightly higher distribution of K_{ow}, with 10% within the class-ranges of hydrophilics, 10% polars, 45% semipolar, and 35% hydrophobics.
- Responsive compounds observed in surface water studies by RIZA exhibit a similar distribution pattern, with slightly lower frequencies in the category of the hydrophilic compounds.
- Compared to the information from Chapter-2 it is important to note that approximately 50% of the UmuC-responsive compounds lies within the critical interval for break-through losses of many commonly used SPE phases.

3.5 Distribution of Henry's Law Constants

Estimated and experimentally derived values for H are given in Annex-4. Values are given in $Pa.m^3/Mol$. Experimental values were available for 39 (24%) of the compounds, of which 27 (17%) were calculated from reliable vapour pressure and solubility data in the SRC PhysProp database. Less reliable predictions with H values below the cut-off value of 10^{-4} ($Pa.m^3/Mol$) have been listed in Annex-4 and will not be treated further in the analysis.

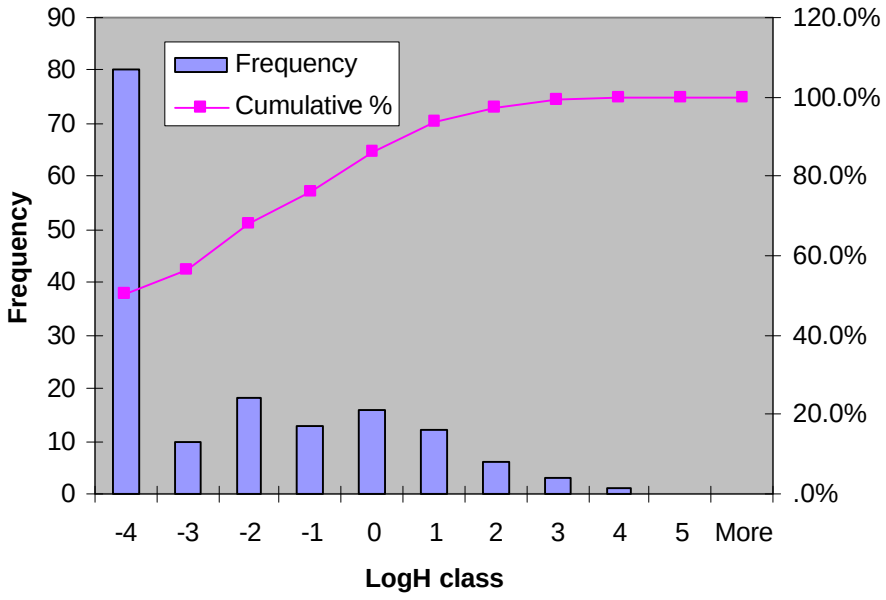


Figure 3.2 Frequency distribution of predicted (n=120) and experimentally derived (n=39) Log H ($Pa.m^3/Mol$) values for UmuC-positive compounds. Compounds below Log H = -3 are involatile from water. Cut-off value for volatiles: Log H = 1.6 (naphthalene)

Frequency distributions of the most important classes are given in Figure 3.2 and Table 3.3.

Table 3.3 Frequency distribution of Log H of UmuC responsive compounds.

LogH class	Frequency	%	Cumulative %
<-4	80	50%	50%
-4 to –3	10	6.3%	56.6%
-3 to –2	18	11%	67.9%
-2 to 0	29	18%	86%
0-1	12	8%	94%
1-2	6	4%	97%
>2	4	3%	100%
Total	159		

Approximately 50% of the responsive compounds have extremely low Log-H values, below the cut-off value of –4. Approximately 56% of the values is below the level for virtually involatiles from water (Log H <-3; Thomas, 1990). The arbitrarily assigned category of low-volatility (Log H >-3 and <-2) comprises 11%. The category of intermediate volatility from water (Log H <-2 to 1) constitutes 24%. Compared to naphthalene (Log H=1.6), which is considered as a relatively volatile PAH, approximately 7% has a similar or higher volatility from water (Log H > 1).

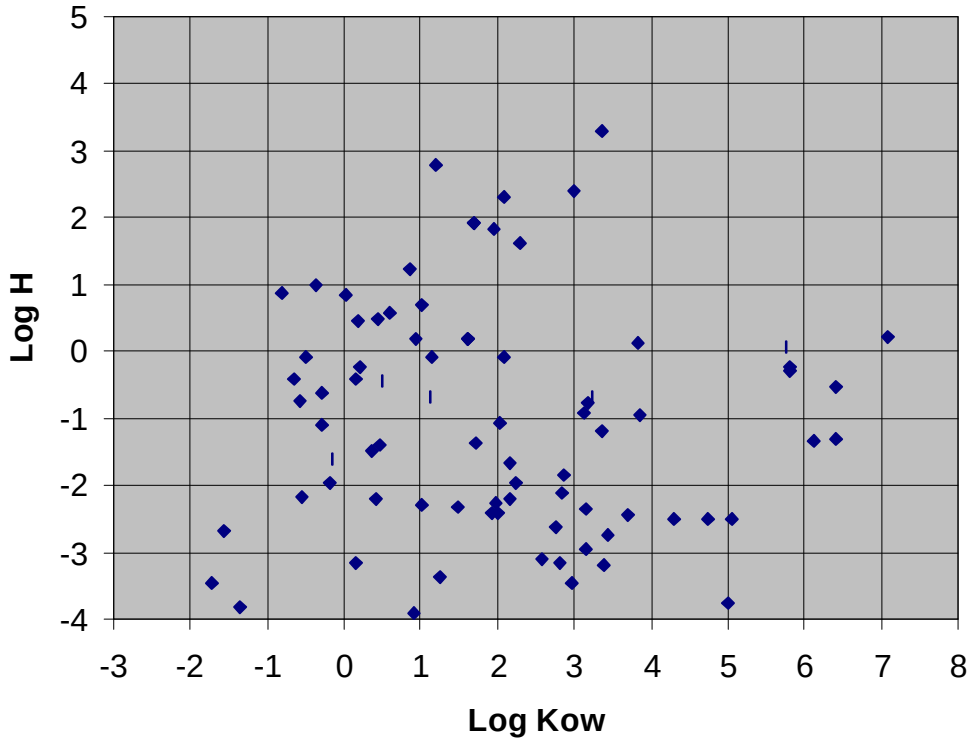


Figure 3.3 Plots of Log H versus Log K_{ow} of UmuC responsive compounds. Only observations with Log H >-4 are included..

Compounds sensitive to volatilisation during sample-handling probably will have a Log H > 0. From the plot in Figure 3.3 it can be derived that the hydrophobicity of these

compounds (n=22) ranges from $LogK_{ow}$ = -3 to $LogK_{ow}$ > 3. In table 3.4 these compounds have been listed individually.

Table 3.4 Selection of UmuC-positive compounds prone to evaporative losses during sample-prep.

Compound	CASnr	LogH	Reference exp. value
Benz(a)anthracene(+ S9)	56-55-3	0.07	Bamford, HA et al. (1999)
Azobenzene(+ S9)	103-33-3	0.12	SRC
Epoxystyrene	96-09-3	0.19	SRC
Styrene oxide	96-09-3	0.19	SRC
t-Butylhydroperoxide	75-91-2	0.20	
Nystatin	1400-61-9	0.21	
1,3-Dioxane	505-22-6	0.46	
Epichlorhydrin	106-89-8	0.47	SRC
4-Methyl-1,3-dioxane	1120-97-4	0.59	
2,4-Dimethyl-1,3-dioxane	766-20-1	0.71	
Propylene oxide	75-56-9	0.83	SRC
8-Proplotactone	57-57-8	0.88	
5-Butyrolactone	36536-46-6	1.00	
1,2-Epoxybutan	106-88-7	1.25	SRC
Benzylchloride	100-44-7	1.61	SRC
Ethylene dibromide	106-93-4	1.82	SRC
Methylene bromide	74-95-3	1.91	Moore, R.M. et al. (1995)
Trichloronitromethane	76-06-2	2.30	Kawamoto,K & Urano, K. (1989)
Bromobenzene	108-86-1	2.38	Shiu,W.Y. & Mackay, D. (1997)
Methylbromide	74-83-9	2.79	SRC
1-Bromo pentane	110-53-2	3.29	SRC

4. Selection of suitable SPE methods

4.1 Introduction

The key problem when applying SPE remains the method development and the primary decision for the analysts is the choice of the sorbent that is able to solve their trace-analysis problem. Method development being related to the properties of the analytes of interest, many aspects due to the various physico–chemical properties of compounds included in a multiresidue analysis have to be considered. A first approach for the method development is the process, which occurs during the extraction and this approach has remained rather empirical based on experimental trial-and-error procedures. Alternative approaches based on computer-aided strategies and simulation require an appropriate level of theory so that at decision steps in the method development process fast simulation or calculation procedures can be used in place of trial-and-error experiments.

For the extraction of compounds from a wide range of polarities, the analyst usually focuses on the low retention of polar analytes, which can be lost during the extraction step. The potential of the highly cross-linked polymers and the carbon-based sorbents for the extraction of (very) polar compounds was previously demonstrated. However, for this type of analysis, difficulties other than those related to the low retention of polar compounds can be expected (see below).

A typical SPE sequence consists of four general steps: (i) conditioning the sorbent, (ii) percolating the sample, (iii) drying the cartridge and (iv) desorption. These steps are described and presented in the following scheme.

In chapter 3, a study of physicochemical properties of the 159 target analytes covering broad ranges of polarity and volatility was presented. Polarity is presented in the form of Log K_{ow} (Log K_{ow} range from – 4 to 6) and volatility in the form of Log H. In general, it is always a challenge to extract as much as possible in one run in order to decrease the cost and the time of the analysis in the environmental field (Pichon et al., 1998 and Nakamura et al., 1996). It depends on the polarity of the most polar analytes and one has to check if these ones are extracted with good recoveries with the required sample volume to be handled. For the hydrophobic ones, in theory, there is no problem of breakthrough.

However, if the extraction can be predicted, very often practical problems coming from the physico-chemistry are encountered. One problem may occur during the sample percolation, because recoveries of hydrophobic analytes with very low water solubility are low unless a certain percentage of organic solvent or surfactant is added in the sample (Pichon et al., 1998 and Nakamura et al., 1996). But if the addition of an organic solvent solves the problem of the hydrophobic ones, it decreases the breakthrough volumes of the more polar ones, which can then ben poorly recovered using this procedure. Another problem is in the reconstitution of the extract. When very polar and non-polar analytes are together, complete solubilization of the extracts is often impossible: addition of water (or methanol) for the more polar ones, whereas very hydrophobic analytes can only be dissolved in a non-polar organic solvent.

Typical steps in SPE sequence

Conditioning solvent (typically 3-5 hold-up volumes)
(a) Ensures reproducible retention and flow. Critical step for particle-loaded membranes
(b) Helps to minimise contamination of extracts by solvent impurities
(c) Replace by sample solvent before processing sample

Flow rates (typical range 0.2-1.5 mm/s)
(a) More critical for cartridges than disks due to their variable and heterogeneous packing density (channelling)
(b) More critical when the sample volume exceeds the breakthrough volume as typical sampling devices provide too few theoretical plates for flow independent retention

Sample proprieties
(a) Remove excessive particle matter by filtration or centrifugation to maintain a constant sample-processing rate
(b) Add small volume of organic solvent (1-10%, v/v) to large volume water samples to ensure sorbent remains solvated and to maintain a constant (fast) sample-processing rate. Critical for polar analytes (see text)
(c) Adjust pH to reduce ionisation of weak acids and bases for reversed-phase sampling

Drying time (typically 1-5 min, but sometimes considerably longer)
(a) Sufficient to remove all sample solvent trapped in the sorbent pores
(b) Excessive drying may result in low recovery of analytes from evaporation or retention in poorly solvated regions of the sorbent

Eluting solvent (ideally 2-3 hold-up volumes but often larger)
(a) Should be a strong solvent able to displace all analytes from the sorbent with a small volume
(b) Normally should be volatile and miscible with sample solvent

4.2 Application of selection criteria

From chapter 2, five possible candidate sorbents have been selected, which have the potential of covering the polarity range mentioned above i.e., -4–6.

Four critical selection criteria were used: breakthrough volume, desorption mixture/volume, recovery and enrichment factor. Other parameters, such as costs, simplicity and ease of implementation were not discriminatory between different SPE methods.

For the sake of clarity, we divided the polarity range into four classes of compounds: A for hydrophilic (Log K_{ow} < 0), B for polar (0 < Log K_{ow} < 1), C for medium polar (1 < Log K_{ow} < 3) and D for hydrophobic (Log K_{ow} > 3).

The scores from ‘-‘ to ‘++’ of each SPE method for each parameter along this compound classification are presented (see Table 4.1). Each parameter of the four critical ones shown as legend is divided into four categories from bad ‘-‘ to excellent ‘+ +’. For instance, breakthrough volume higher than the minimum selected volume (500 ml) is considered as excellent and lower than 5 ml is bad. Some scores shown in the Table like ’+/-‘, means that the results vary between the two scores, and that is because either the Log K_{ow} is relatively large and/or the behaviour may depend on other experimental conditions and physico-chemical properties of compounds.

Three parameters are important to achieve a desired enrichment factor, i.e., breakthrough volume, desorption characteristics (mixture/volume dimensions), and recovery. Other aspects such as simplicity, costs and implementation are not discriminative regarding the final selection of an SPE method (see Table 4.2). The basic steps of the SPE procedure

are simple and straightforward and can be trained in a one-day course. All equipment can be purchased from various sources and costs differ mainly in the degree of automation, which can vary from single-sample off-line procedures up to fully automated batch processing. Drying of cartridges is only discriminative when methylene chloride is used as desorption solvent. The problem is, especially for carbon cartridges, that water remains in the pores of the stationary phase and that methylene chloride is not able to reach the analytes, which leads to inefficient desorption.

Table 4.1. Criteria table for the five selected sorbents.

SPE method	Breakthrough volume[1]	Desorption[2]	Recovery[3]	Enrichment Factor[4]	Drying (cartridge)
Lichrolut EN	A: - / ±	A: + +	A: - / ±	A: - / ±	
	B: +	B: + +	B: +	B: +	+ / ±
	C: + +	C: + +	C: + +	C: + +	
	D: + +	D: + / -	D: + / ±	D: + / -	
Isolut ENV+	A: - / ±	A: + +	A: - / ±	A: - / ±	
	B: +	B: + +	B: +	B: +	+ / ±
	C: + +	C: + +	C: + +	C: + +	
	D: + +	D: + / -	D: + / ±	D: + / -	
Oasis HLB	A: -	A: -	A: -	A: -	
	B: + / ±	B: +	B: +	B: ±	+
	C: +	C: +	C: +	C: ±	
	D: +	D: +	D: +	D: ±	
C18	A: -	A: + +	A: -	A: -	
	B: -	B: +	B: -	B: -	+
	C: ±	C: +	C: ±	C: ±	
	D: + +	D: + +	D: + +	D: + +	
GCB	A: +	A: + +	A: +	A: +	
	B: + +	B: +	B: + +	B: + +	-
	C: + +	C: ±	C: + +	C: + +	
	D: +	D: -	D: - / ±	D: +	

Explanation

A. Log K_{ow} < 0	+ +	= excellent
B. 0 < Log K_{ow} < 1	+	= good
C. 1 < Log K_{ow} < 3	±	= intermediate
D. Log K_{ow} > 3	-	= bad

Scores

[1]Breakthrough (ml)		[2]Desorption (ml)		[3]Recovery (%)		[4]Enrichment factor	
+ +	= 500	+ +	= < 2	+ +	= > 85	+ +	= > 1000
+	= 100–500	+	= 2–5	+	= 50–85	+	= 100–1000
±	= 5–100	±	= 5–10	±	= 5–50	±	= 10–100
-	= < 5	-	= > 10	-	= 0–5	-	= < 10

The groups presented here are derived from Annex A1 (factsheets of selected SPE methods) directly or indirectly as follows: target compounds present in Annex A1 are directly included. If one or a group of target compounds are missing, then comparison and estimation are made according to their physico-chemical properties.

The following example should clarify the table. LiChrolut EN can be used in the Log K_{ow} 0–3 range efficiently (B: +; C: ++). Outside this range, two problems appear. The first one is the desorption problem, especially for very hydrophobic analytes (D: +/-). The second one is recovering the more polar ones. To solve the first problem, a com-

promise is needed. Addition of a miscible organic solvent to the sample (see below) before sampling can solve this problem but there is risk to loss (very) polar analytes. That is why a '+ / -' scores is given for the desorption of hydrophobic compounds from LiChroliut EN sorbent. The same selection strategy was used for the rest of the Table.

In Table 4.2 the scores for the non-selective criteria are indicated. Ease of handling, handling time required for the procedure and potential for implementation in routine laboratory settings do not differ much between the different methods. Disk-options usually are more rapid compared to cartridges. As personal costs are dominant, the slight differences in price between disks and cartridges are of minor influence.

Table 4.2 Non-selective criteria.

SPE method	Simplicity	Costs	Implementation
Lichrolut EN	+	+	+
Isolut ENV+	+	+	+
Oasis HLB	+	+	+
C18	+	+	+
GCB	+	+	+

4.3 Constraints from the UmuC-test protocol

In order to be able carry out the UmuC-test successfully, the concentration technique has to meet some requirements. First, enrichment factor must be high enough (1000 or higher) to achieve the sensitivity needed. A sample volume of 500 ml or higher is needed to be handled and to get a final extract volume of 0.3–0.5 ml. Second, the final extract solvent must be suitable for the UmuC-test. Methanol, acetone and DMSO (Rotteveel, personal communication) are tolerable solvents at 3% volume for the test (water is the ideal solvent if possible). Finally, to improve the enrichment factor and to avoid loss of volatiles during evaporation, a high boiling point solvent (DMSO or water) can be added to the extract.

4.4 Various SPE approaches

As can be seen in Table 4.1, each sorbent shows its advantages and disadvantages along the four Log K_{ow} ranges. It is very difficult to find a single method to extract the polar and non-polar analytes together in one procedure. Highly cross-linked polymers are capable to extract polar and moderately polar analytes efficiently. For the very polar (Log $K_{ow} < 0$), GCB is recommended. However, other kind of problems appears; compounds with low water solubility cannot be recovered. This can be due to adsorption of these compounds on tubings and vessels or to an incomplete desorption of strongly retained compounds on the sorbent. The difficulty to simultaneously extract compounds from a broad range of polarities was well described by Norberg et al. (1995) in the case of the study of organophosphorus compounds. When plotting the recoveries vs. the sample volume, three types of behaviour were observed. For the most polar compounds, a quantitative recovery was found for low sample volumes (early breakthrough). For the moderately polar compounds, recoveries were quantitative over the whole 10-100 ml range. For the most hydrophobic analytes, recoveries slowly increased with the sample volumes

because a fraction of the analytes was adsorbed in the preconcentration system. To eliminate adsorption phenomena, a low percentage of organic solvent (1-10%, v/v) or a low amount of surfactant can be added directly to the sample (Pichon et al., 1998 and Nakamura et al., 1996). However, the presence of these modifiers decreases the retention of polar compounds on the sorbent during the percolation and causes loss of these compounds. Therefore, a compromise has to be found between adsorption of the low water-soluble compounds and the retention of the highly polar ones.

Concerning the problem of incomplete desorption, especially for GCB cartridge, it can be difficult to find the solvent that will allow a complete desorption in a small volume in order to obtain a high enrichment factor. In case of large fraction, it can be reduced by evaporation of the residue but this step needs to be well controlled because of the risk of loss of high vapour pressure compounds, i.e., phenols (Castillo et al., 1997) or organophosphorus compounds (Molina et al., 1996). In most cases, in order to limit the losses of volatile compounds, the evaporation step is carried out under mild conditions, i.e., under a stream of nitrogen instead of the use of a rotary evaporator until a final volume of 0.5 ml. Besides, a degradation of compounds in contact with some solvents can occur during this step as it was mentioned for aryloxyphenoxypropionic acids (Lagana et al., 1998).

Many studies reported the difficulty to select proper solvents (Castillo et al., 1997 and Di Corcia et al., 1993). As an example, methanol was preferred above acetonitril for the desorption of catechol from PS-DVB sorbent (LiChrolut EN) owing to a better solubilisation of this compound with methanol. The authors recommended a mixture of methylene chloride-methanol (4:1) according to their eluting strength on reversed-phase sorbent. It was also pointed out that residual water in the GCB cartridge had to be reduced to a minimum and, that, when this was not done, low and irreproducible recoveries were obtained because the water can hinder intimate contact between the desorption mixture and the GCB.

In the case, that the range of polarities is too broad, two separate procedures are recommended, one optimised for the (very) polar and moderately polar ones, and in a subsequent suited to the non-polar ones (Pichon et al., 1998 and Nakamura et al., 1996). When two subsequent procedures are required, it can also be interesting to use two different sorbents to facilitate the desorption. As an example, two off-line procedures were defined: (i) the polar and moderately polar pesticides were extracted on a PS-DVB disk and subsequently eluted with acetonitrile (methanol is also possible), (ii) after the addition of 10% of methanol to the sample, the non-polar pesticides were extracted on a C_{18} disk and eluted with a mixture of methylene chloride-methanol (4:1) (Pichon et al., 1998). However, in this study, a Log K_{ow} 1-6 range was considered and very polar analytes were not included. Very polar analytes may still be difficult to extract with these sorbents, and graphitized carbon has been shown to solve this problem for some analytes (Pichon et al., 1995; Hennion et al., 1995; and Guenu et al., 1996). For hydrophobic compounds, a C_{18} silica is usually preferred over PS-DVB sorbent since recoveries are good for a sample volume of 500 ml or higher even after the addition of methanol before the extraction step.

Castillo et al. (2001a; 2001b; 1999a-c); Thomas et al. (2001), La Farre et al., 2001a-d) and Remstsma et al. (1999) used a sequential SPE (SSPE) method to extract polar and

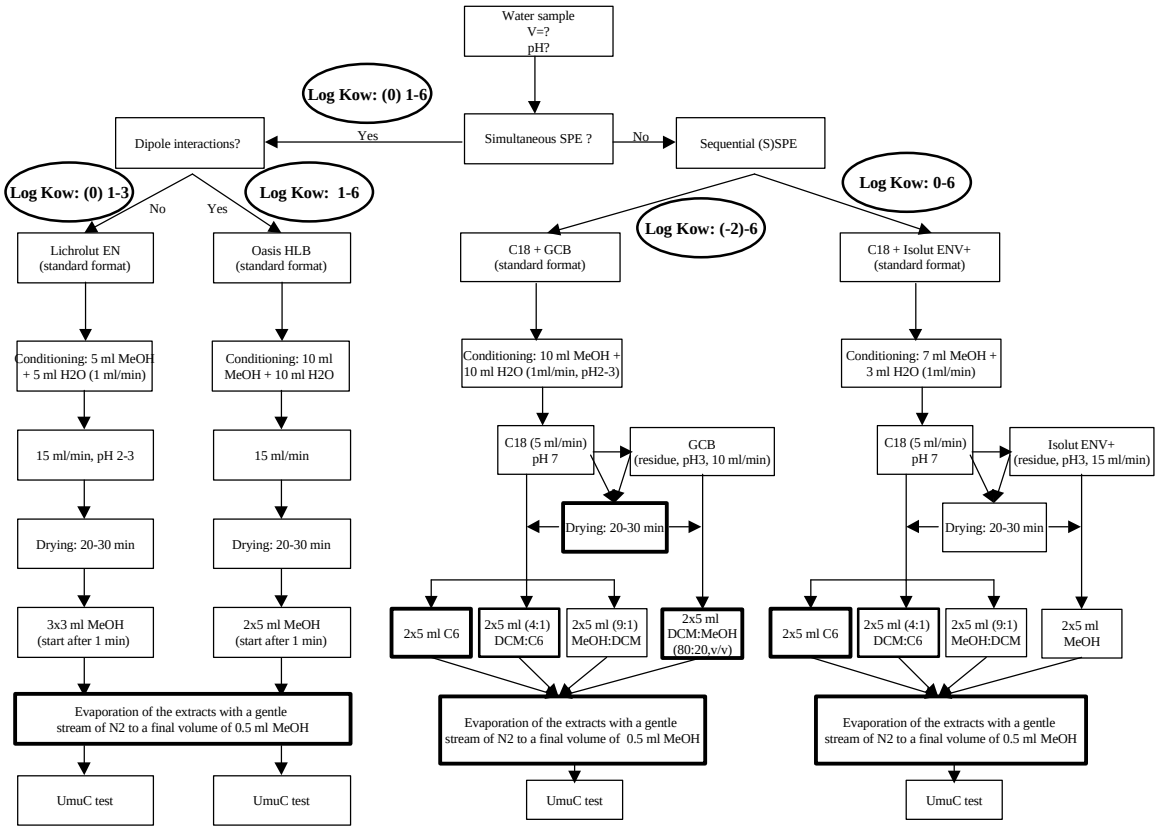
non-polar analytes by two separate procedures (C_{18} silica and Isolut ENV+ or LiChrolut EN). This method was used in order to extract non-polar compounds on C_{18} silica and moderately polar and polar ones on PS-DVB polymer (Isolut ENV+) (for more details, see Figure 4.1).

Putschew et al. (2001) used the same SSPE approach but with LiChrolut EN (200 mg) and Envi-Carb (250 mg) cartridges in order to extract the very polar and partly ionic analytes. Recoveries of the eight selected very polar iodinated benzene derivatives were in the range of 80–100%. The enrichment factor was higher than 1000. It was not possible to achieve good recoveries for all compounds by just one extraction step. The only drawback of this method is that the Envi-Carb cartridge had to be eluted against the extraction direction (back flush) with a mixture (8 ml) of acetonitrile-ultra pure water (1:1, v/v) and trace of ammonium acetate.

It is clear from Table 4.1 and the above discussed parameters that the conditions for SPE must be carefully selected to achieve the goal described in the Introduction. Considering the physico-chemical of the components (Log K_{ow}) and the characteristics of the sorbent, the guidelines for the selection of SPE conditions shown in Figure 4.1 should be useful. Although, from this figure it might appear that the combined procedures are complicated, the contrary is true. These procedures are consist of two simple SPE procedures, although carried out in a sequence.

When very polar (Log $K_{ow} < 0$, i.e., 35 from the 170-component list) are not included in the compound list, the SSPE procedure using C_{18} silica and Isolut ENV+ in series is recommended. That is because no desorption problems occur and the addition of methanol (up to 10%) is possible by C_{18} silica. Moreover, good recoveries (higher than 80%) and high enrichment factors (higher than 1000) can be obtained for all compounds over Log K_{ow} 0–6 range. A compromise is needed when very polar (Log $K_{ow} < 0$) analytes are added to the list. Using C_{18} silica and GCB cartridges in series can recover the Log K_{ow} – 2 to 6 range but some desorption problems by GCB can be expected and sometimes a backflush desorption is necessary. These desorption problems were already obviously encountered with the GCB cartridges: pure methanol, acetonitrile or dichloromethane were unable to desorb many organic pollutants. With GCB cartridges, the desorption of many analytes occurred with 6 ml of dichloromethane: methanol (80:20,v/v) with 300 mg of sorbent and the backflush desorption is even recommended for the 1-g cartridges. Cartridges allowing percolation and desorption in the opposite way are now available.

Oasis HLB sorbent is selected because it is capable of extracting acidic, basic and neutral compounds whether polar or non-polar. Retention of some analytes measured on a column packed with this polymer was compared with that obtained on C_{18} silicas, showing a large increase of Kw values. For instance the Log Kw value for catechol is around 2.5 whereas it is 1.1 on C_{18} silica. It was measured to 1.6 using PRP-1. The increase in retention is explained by both the specific area of 800 m^2/g and the occurrence of the pyrrolidone group in the polymer, which is a hydrogen acceptor (dipole interactions). It is a new sorbent so that more work should be done to look at the limitation in the extraction of (very) polar analytes as it was done for other highly cross-linked PS-DVB polymer. To date, Oasis HLB is more used to extract drugs from plasma and it is not recommended when high enrichment (higher than 200) factor is required.



Abbreviations: C18: silica cartridge; GCB: carbon cartridge; H2O: water; C6: *n*-hexane; DCM: dichloromethane; MeOH: methanol; N2: gas nitrogen.

Figure 4.1. Flow diagram of the selected SPE procedures. Parts presented in bold are the most critical steps in each SPE procedure (see text for more details).

4.5 Proposal

The following conclusions are made as a result of a number of carefully selected studies on this field by the authors and members of the board group. The major difficulty in the environmental sciences is the separation/detection of compounds that are present in the environment at low concentrations (typically µg/l level). An efficient concentration technique is required. SPE is the method of choice over other discussed methods. Next to its simplicity, efficiency and flexibility, SPE meets the UmuC-test requirements.

For the extraction of compounds varying over a wide rage of polarities (Log K_{ow} -2 – 6 range), a single SPE procedure should ideally be applied and all the compounds should be eluted in a single fraction prior the UmuC-test. However, difficulties other than those related to the low retention of polar compounds oppose this ideal. In general, extraction of polar and hydrophobic compounds simultaneously is rather difficult due to desorption problems. In case of quantitative extraction of polar compounds, desorption of the (most) hydrophobic is nearly impossible.

To overcome this dilemma, a sequential SPE procedure is recommended of which a part is optimised for the (very) polar and moderately polar compounds, and the other one suits to the non-polar ones. A combination of two different sorbents is preferred to facilitate the desorption. C_{18} silica and PS-DVB (Isolut ENV+) can be used efficiently in series to recover Log K_{ow} 0 – 6 range. The hydrophobic compounds are extracted on the modified C_{18} silica after addition of 10% of methanol to the sample and eluted with a mixture of methanol–methylene chloride (9:1). Next, the polar and moderately polar compounds present in the residue from the C_{18} cartridge, are extracted on the PS-DVB sorbent after pH adjustment of the residual sample, and subsequently eluted with methanol.

When very polar analytes (Log K_{ow} < 0) are added in the list, GCB can solve the problem and can be combined with modified C_{18} silica in a similar sequential SPE procedure. The only difference is that analytes are eluted with a mixture of methylene chloride–methanol (4:1) from the GCB material. The only drawback of this method is that sometimes desorption problems by GCB occur so that a backflush desorption mode is necessary.

In the final step, the desorption solvent is evaporated under a gentle stream of nitrogen to a volume of 100–500 µl depending on the required enrichment factor. In this step, all traces of methylene chloride are removed.

The detailed descriptions of the SPE approaches in Figure 4.1 are presented in Annex-5.

5. Discussion and concluding statements

From the currently available method for enrichment of contaminants SPE methods have been selected as the method of choice. They meet the main requirements for the final UmuC-testing, i.e. an enrichment by a factor of thousand and compatibility with the solvent requirements for this test. Although simple procedures such as lyophilization, reverse osmosis and freeze drying and with a fully compatible extract, viz water, their limitations exclude them for further investigations.

With the current state-of-the-art in SPE techniques it was not possible to select one single generic method, suitable for routine (waste)water screening by the UmuC-test, which is fully comprehensive. For the group of organics with extremely high or low hydrophobicity, special dedicated single-step methods have to be applied. In both cases, recovery at one end automatically leads to losses at the other end. If the polarity range is more limited, several newly developed SPE materials are available, especially for these compounds having log K_{ow} values in the 0 – 6 range. If the range of compounds is limited to the more polar end of the scale, GCB materials can be selected. Generic experimental conditions are described in a special fact sheet in Annex 5.

From the combination of figures 2.1 and 3.1 it becomes clear that most commonly used SPE materials exhibit breakthrough losses in $LogK_{ow}$ interval of 0-3, which coincides with $LogK_{ow}$ of more than 50% of the UmuC-responsive compounds.

The proposed SSPE method covers a wide range ($LogK_{ow}$:-2 to 6), but probably 10% of the compounds in the hydrophilic range (<-2) and approximately 5% in the superhydrophobic range (>6) will be missed. Further losses due to evaporation during sample-handling may be present for approximately 14% (n=22) of the compounds, if a conservative cut-off of H=0 is chosen. From the plots in Figure 3.3 it can be derived that these compounds do not coincide with the superhydrophobics or super hydrophilic compounds, and that losses up to 30% of the total range of compounds may occur. If the volatile fraction is of importance, than the additional application of purge-and-trap concentration techniques prior to genotoxicity testing, may reduce the number of ‘missed’ compound with more than 10%.

The category of inorganic compounds has been excluded in this study. Although in theory adding specific ion-exchange resins in sequence with the other SSPE may result in the trapping of metallic compounds of interest (e.g. selenium, chromium compounds), it is not possible to isolate the redox-state and chemical species, which were tested as genotoxic. Usually these reactive species are only a fraction of the total element concentration in (waste)water. Inorganic compounds which are considered as genotoxic, usually were tested as pure solid compounds, representative for exposure in occupational conditions (e.g. paint and semi-conductor factories), and to a lesser extent as dissolved compounds. Based on a rough comparison of reported LEDs (lowest effective dose) and HIDs (highest ineffective dose) for Se and Cr compounds in the IARC/EPA-GAP database, exposure levels (mg/L to g/L) by far exceed levels commonly encountered in effluents of wastewater treatment installations. Against this background, we recommend not to include inorganics in the generic screening approach. In cases where genotoxicity of

inorganics may be of importance (e.g. effluents of specific industrial sectors), dedicated studies are considered as more cost-effective.

In the scheme in Figure 4.1, two sequential-SPE procedures are presented, that can be used to extend the polarity range. Addition of some 10% of MeOH to the sample excludes losses of hydrophobic analytes. Compounds not retained in the first SPE cartridge (C18) are subjected to a second stationary (more tententive) phase (GCB or PS-DVB). After drying an elution, both extracts are recombined and concentrated further by a gently nitrogen purge. If the toxicity has to be evaluated by means of identification of analytes, scheme 4.1 also proposes procedures to yield separate fractions (with 4 intervals) for these TIE-studies

In previous work, commissioned by RIKZ, detailed procedures have been documented (Adahchour et al., 2000a and 200b). Several of the indicated sorbent materials can also be incorporated in a HPLC setting, for on-line separation in combination with automated fraction-collecting systems, dosing to e.g. microwell-plates suitable for further testing with the UmuC-protocol, similar as the Oasis-based system (Tox-Print) described by Bobbeldijk et al. (2001).

In conclusion, the present study revealed that genotoxic compounds exhibit orders of magnitude variations in volatility and hydrophobicity. From the currently available concentration techniques SPE/SSPE techniques were assigned as the method of choice for the screening (waste)water and combination with the UmuC-test. A wide range of SPE materials, documented in factsheets is commercially available. The most critical parameters for the proper selection of sorbent materials and dimensions are: volatility, breakthrough volume and desorption characteristics. A combine SSPE method, including C-18 followed by GCB was proposed as the most comprehensive method for contaminants with Log Kow ranging from –2 to 6.

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Annex section

List of Annex Tables

A1. Factsheets of SPE techniques

A2. UmuC responsive compounds included in the survey, occurence in surface water surveys by RIZA

A3. Predicted and measured values for Kow

A4. Predicted and measured values for H

Annex 1 Factsheets

Example and explanation of information provided in the factsheets.

0 *Material*	Extraction technique **(SPE) material used (supplier)**
Amount material (mg) and size	Amount of (SPE) sorbents and size of the used cartridges
Deactivation	Conditioning of the (sorbent) cartridge
Kind of sample (ml)	Amount and kind of the analysed water samples
Breakthrough volumes (ml)	Are there breakthrough volumes reported for analytes under the used method and conditions?
(Amount) eluent (ml)	Amount and kind of desorption solvent
Fraction (nr.)	Hoe many fractions are there after desorption?
Drying method	Hoe is the obtained eluate further evaporated (enriched)?
Enrichment factor	Which enrichment factors are obtained?
Compounds	Which (target) compounds are analysed?
Recovery	Under the above conditions what are the recoveries for these compounds?
Repeatability	Is the method repeatable?
LOD's	What are the Limit Of Detections (LOD) obtained by this method
Reference	Article
Remarks	Additional remarks, which can be relevant for the selection of (SPE) extraction method.

1	Sequential SPE (SSPE)
Material	**C18 + Isolute ENV+/LiChrolut EN in serie**
Amount material (mg) and size	C18 (500 mg, 6 ml) + LiChrolut EN/Isolut ENV+ (200 mg, 6 ml)
Deactivation	7 ml MeOH + 3 ml water (1 ml/min)
Kind of sample (ml)	all 200 ml (filtered if needed) of raw tanner wastwater three wastwater (effluent) wastewater industrial effluent drinking water
Breakthrough volumes (ml)	?
(Amount) eluent (ml)	1. 2x5 ml hexane 2. 2x5 ml CH2Cl2/hexane (4:1) 3. 2x5 ml methanol/CH2Cl2 (9:1) 4. 1 ml water (5mM TEA + 5mM acetic acid, pH6.5) 5. 9 ml methanol
Fraction (nr.)	1, 2, 3, 4, 5
Drying method	Under gentle stream of N2
Enrichment factor	Up to 1000
Compounds	Linear alkyl benzene-sulfonate (LAS) + Non-ionic polyethoxylated surfactant (PEO) + benzene-and naphthalene-sulfonates (BS and NPS) C10LAS; C11LAS; C12LAS; C13LAS; 1,5-NPDS; 2,6-NPDS; 2,7-NPDS; 1-hydroxy-3,6-NPDS; 1-amino-5-NPS; 1-amino-6-NPS; 1-amino-7-NPS; 1-hydroxy-1-amino-3-NPS; 3-nitro-BS; 1-hydroxy-4-NBS; 4-methyl-BS; 4-chloro-BS; 1-NPS; 2-NPS; 1-amino-6-NPS; 1-amino-7-NPS; catechol; phenol; 4-methylphenol; 2,4-dinitrophenol; 2,2'-dihydroxybiphenyl; pentachlorophenol; naphthol; poly(ethylene glycol); nonylphenol polyethoxylate; alcohol ethoxylate; dibutyl phthalate; dimethyl phthalate; bis-2-ethylexyl phthalate; tributylphosphate; ethylbenzoate; 1-methyl-2-pyrrolidinone; 2-methylbenzenesulfonamide; 2,2'-dimethyl-1,3-propanediol; 4-chlorophenol; 2-chlorophenol; 2,4-dichlorophenol; 2,4,6-trichlorophenol; pentachlorophenol; Isoproturon; diuron; simazine; atrazine; 4-chloro-m-cresol;
Recovery	72-103%
Repeatability	1-11%
LOD's	Low ppb level
Reference	M. Castillo et al./ Analytica chimica acta 426 (2001) 265 M. Castillo et al./ Waste Managment 19 (1999) 101 M. Castillo et al./ Analytica chimica acta 426 (2001) 253 M. Castillo et al./ Trends in Analytical Chemistry 18 (1999) 26 M. Castillo et al./ Analytical Chemistry 71 (1999) 3769 K.V. Thomas et al./ Wat. Res. 35 (2001) 2411 M. la Farre et al./ Analytica Chimica Acta 426 (2001) 155 M. la Farre et al./ Analytica Chemica Acta 427 (2001) 181 Th. Reemtsma et al./ Waste Management 19 (1999) 181
Remarks	The developed methodology with toxicity measurements

allowed to detect different groups of pollutants responsible for the toxicity of the studied wastewaters. This method can cover broad log Kow range (0-6) efficiently and no problems are reported compared to other methods (GCB).

2	SPE
Material	**Lichrolut EN (Merck)**
Amount material (mg) and size	200 mg
Deactivation	5 ml methanol + 5 ml HPLC-grade water
Kind of sample (ml)	1000 for atrazine 100 for degradation experim.
Breakthrough volumes (ml)	?
Amount eluent (ml)	3x3 ml methanol
Fraction (nr.)	1
Drying method	evaporated to 0.5 under N2
Enrichment factor	200–2000
Compounds	atrazine, hydroxyatrazine, desethylatrazine, desisopropylatrazine
Recovery	?
Repeatability	?
LOD's	?
Reference	R.J.C.A. Steen et.al./ J. chromatogr. A 915 (2001) 129
Remarks	The rather polar transformation products (TPs) are retained on the sorbent used (LiChrolut EN). Fortunately, this material has already been shown to be a good choice to retain relatively polar TPs and even partially ionized compounds (if necessary, after pH adjusment)

3	SPE
Material	**Isolute ENV+ (International Sorbent Technology, Ltd)**
Amount material (mg) and size	200 mg, 850 Å pore size, 1100 surface area, 1 ml cartridge
Deactivation	MeOH, C6, acetone (9 ml for each solvent) and 30 ml of Milli-Q water
Kind of sample (ml)	4–5 l filtered surface water samples by pH 7 (first column) and then the same procedure by pH 2 (second column) (30–40 ml/min flow rate)
Breakthrough volumes (ml)	-
Amount eluent (ml)	9 ml of C6/acetone (85/15,v/v) followed by 6 ml of MeOH
Fraction (nr.)	2
Drying method	The obtained extracts from the two columns (pH 2.0, 7.0) were combined and the volume was reduced using a Kuderna–Danish evaporator with atmospheric air and a temperature of 30ºC.
Enrichment factor	300–1500
Compounds	Not reported (only toxicity is studied)
Recovery	-
Repeatability	-
LOD's	-
Reference	A. Baun et al./Environmental Poluttion 102 (1998) 185
Remarks	

4	SPE
Material	**Oasis (Waters)**
Amount material (mg) and size	?
Deactivation	?
Kind of sample (ml)	500 surface water filtered over 0.2 um
Breakthrough volumes (ml)	?
Amount eluent (ml)	4x2 ml acetonitrile (after 1 ml of 5% acetonitrile in water and 30 min drying with N2 evaporated to 0.5 ml under N2
Fraction (nr.)	1
Drying method	-
Enrichment factor	1000
Compounds	metribuzine, pirimicarb, diuron, isoproturon, metamitron, atrazine
Recovery	-
Repeatability	-
LOD's	lower than 0.25 ul/l
Reference	I. Bobeldijk et. Al./ j. chromatgr. A 929 (2001) 63
Remarks	Further research concerning alternative solvents, isolation, overall sensitivity and other parameters is needed to overcome the matrix and other interference effects.

5	SPE + HPLC
Material	**Oasis (Waters) + pellicular C18 guard column + inertsil ODS-2 analitical Column**
Amount material (mg) and size	?
Deactivation	ultrapure water + acetonitril (gradient, 181 ml)
Kind of sample (ml)	all filtered over 0.2 um 100 ml surface water 100 ml municipal water 20 ml industrial water 100 ml hospital water
Breakthrough volumes (ml)	?
Amount eluent (ml)	58 ml linear gradient of acetonitrile and water (58 ml)
Fraction (nr.)	(1 min fraction) and fractions 11-46 are collected
Drying method	-
Enrichment factor	-
Compounds	4-nitroquinoline-N-oxide and 2-aminoanthracene (test compounds)
Recovery	75-125%
Repeatability	?
LOD's	low ug/l range
Reference	I. Bobeldijk et. Al./ j. chromatgr. A 918 (2001) 277
Remarks	Oasis sorbent with an adsorption capacity for both lipophilic and hydrophilic compounds was experimentally shown to be capable to extract compounds with a braod range of polarities.

6	SPE
Material	**SDB (Baker)** **Oasis HLB (Waters)** **Envicarb (Supelco)** **Carbograph (Alltech)**
Amount material (mg) and size	200 200 500 150
Deactivation	all cartridges 2x 5 ml desorption solvent, 2x 5ml methanol and 2x 5ml demi-water
Kind of sample (ml)	100 ml surface water
Breakthrough volumes (ml)	Yes!! (see recovery)
Amount eluent (ml)	2x5 ml ethyl acetate 2x5 ml ethyl acetate 2x5 ml ethyl acetate DCM/MeOH (8:2) DCM/MeOH (8:2)
Fraction (nr.)	1, 1, 1, 1
Drying method	?
Enrichment factor	1000
Compounds	Acephate, methamidophos, omethoate, oxydemeton-methyl vamidothion
Recovery	acceptable recoveries for last two, but the first three (more polar) low recoveries are obtianed
Repeatability	better than 20%
LOD's	0.01-0.03 ug/l deponding on the analyte
Reference	B.A. Ingelse et.al./ j. chromatogr. A 918 (2001) 67-78
Remarks	Very polar (Log Kow < 0) OPs like acephate, metamidophos and omethoate can not be extracted from water using the currently commonly available SPE cartridges. But Lichrolut RP-18, Lichrolut EN and Isolut cartridges were not tested.

7	SPE
Material	**1. Lichrolut EN (Merck) and 2. Oasis (Waters)**
Amount material (mg) and size	1. 200 2. 300
Deactivation	1. Hexane, acetone, HPLC water pH2 (6, 6, 6 ml res.) 2. Methanol, HPLC water (6, 6 ml resp)
Kind of sample (ml)	1. 1 l of filtered groud water (pH2) 2. 1 l of filtered ground water (washing with 1ml 5% MeOH in water
Breakthrough volumes (ml)	?
Amount eluent (ml)	1. Acetone, methanol, acetone (1, 2, 2 ml resp.) 2. CH3CN:MeOH(70:30), CH3CN:MeOH(70:30) [3 and 2 ml resp.(solvents acidified to pH 3.7)]
Fraction (nr.)	1. 1 2. 1
Drying method	1. Gentle stream of N2 to 100 ul and then reconstituted with MeOH to 300 ul. 2. Gentle stream of N2 to 100 ul and then reconstituted with 500 ul formate buffer
Enrichment factor	
Compounds	salicylic acid, ketoprofen, naproxen, diclofenac-Na, ibuprofen and gemfibrozil
Recovery	1. 76, 69, 83, 85, 91, and 70 (pH2) 2. <10, 108, 125, 38, and 109 (pH7)
Repeatability	1. 13, 15, 10, 6, 11, and 6 (%SD) 2. Nd, 4.8, 11.6, 24, 12.1 and 2.54 (%SD)
LOD's	1. 15, 28, 29, 5, 43, and 56 (ng/l, SIM mode) 2. Nd, 53, 52, 37, 123 and 72 (ng/l, SIM mode)
Reference	M. la Farre et.al./ J. Chromatorgr. A 000 (2001)000
Remarks	The SPE methode (using Lichrolut-EN) was applied to 12 surface water sample and three effluent samples. While pharmaceuticals are present, and their concentrations increase with observed toxicity, their contribution to the whole toxicity cannot be determined because these substances are acting as tracers of toxicity.

8	SPE
Material	**1. Isolute ENV** **2. LiChrolut EN (Merck)** **3. LiChrolut RP-18 (Merck)** **4. RP-18 (Baker)** **5. Oasis HLB (Waters)** **6. HySphere-resin-GP (Spark Holland)** **7. PLRP-S (Polymer Labs)**
Amount material (mg) and size	1. 500 mg, 3 ml 2. 200 mg, 3 ml 3. 500 mg, 3 ml 4. 10 mm x 2 mm 5. 10 mm x 2 mm 6. 10 mm x 2 mm 7. 10 mm x 2 mm
Deactivation	1. CH3CN, MeOH and LC-grade water (7, 5 and 5 resp.) 2. CH3CN, MeOH and LC-grade water (7, 5 and 5 resp.) 3. CH3CN and LC-grade water (4 and ml resp.) 4. CH3CN and LC-grade water (4 and ml resp.) 5. CH3CN and LC-grade water (4 and ml resp.) 6. CH3CN and LC-grade water (4 and ml resp.) 7. CH3CN and LC-grade water (4 and ml resp.)
Kind of sample (ml)	50 to 1000 ml filtered water samples
Breakthrough volumes (ml)	extraction of large volumes of sample (1000ml), HySphere-Resin-GP and Lichrolut cartridges are the best
Amount eluent (ml)	2x4 ml acetonitrile (alle cartridges)
Fraction (nr.)	1 fraction (each cartridge)
Drying method	all extracts were blown down to dryness under N2 and reconstituted in MeOH to 0.5 ml.
Enrichment factor	
Compounds	Estriol, estradiol, norethindrone, ethynyl estradiol, estrone, levonogestrel, diethylstilbestrol and progesterone.
Recovery	Lichrolut RP-18 and HySphere-Resin-GP give the best recoveries (87-101%)
Repeatability	< 25% (RSD)
LOD's	1-20 ng/l
Reference	M. J. Lopez de Alda et.al/ J. Chromatogr. A 000(2001) 000
Remarks	the first three cartridges (LiChrolut RP-18, 500 mg was better than LiChrolut EN, 200 mg and Isolut ENV, 500 mg) were used in off-line mode and the last four in on-line mode. In general, on-line mode gives the best results.

9	SPE
Material	**1. Lichrolut EN (Merck)** **2. Lichrolut RP-18 (Merck)** **3. Mix. Of the two**
Amount material (mg) and size	1. 200 mg, 3 ml 2. 500 and 1000 mg, 3 ml 3. 100 mg of EN and 100 mg of RP-18
Deactivation	1. 3 ml MeOH 2. 3 ml H2O 3. 3 ml MeOH, 3 ml H2O and 3 ml H2O (pH 4)
Kind of sample (ml)	1 l tap water (pH 4-6) spiked with 33-multicomponent pesticide standard.
Breakthrough volumes (ml)	not observed by 1 l water
Amount eluent (ml)	1. And 2. 2x3 ml MeOH:EtOAc(1:1) 3. 2x3 ml MeOH
Fraction (nr.)	each cartridge gives 1 fraction
Drying method	extracts were blown down to dryness and reconstituted in 1 ml of a mixture of ACN and amonium acetate (20:80)
Enrichment factor	> 1000
Compounds	Deisopropylatrazine, metamitron, chloridazon, deethylatrazine, crimidine carbetamide, bromacil, simasine, cynazine, deethylterbutylazine, karbutilate, metha-benzthiazuron, chlortoluron, atrazine, monolinuron, isoproturon, diuron, metobromuron, metazachlor, methoprotryne, dimefuron, sebutylazine, propazine, terbutilazine, linuron, chloroxuron, pro-metryne, chlorpropham, terbutryne, metolachlor, pencycuron, bifenox, pendimethaline.
Recovery	The best recoveries were obtained by LiChrolut EN (200 mg, 3 ml) and ranging from 81 to 118%.
Repeatability	0.1-13.2% (RSD)
LOD's	0.007 mg/l (S/N ratio of 4:1)
Reference	A.Junker-Buchheit et. Al/ J. Chromatogr. A 737 (1996) 67
Remarks	LiChrolut (200 mg, 3ml) was the best. One gram of LiChrolut RP-18 is not sufficient to achieve quantitative recoveries of the most polar components (low Log Kow). This problem was circumvented by increasing the mass of the sorbent (up to 2000 mg). The method was applied to the analysis of drinking water and surface water. Problems arising from high contents of humic acid compounds were circumvented by using a mixed RP-EN stationary phase.

10	SPE
Material	**1. LiChrolut RP-18 (Merck)** **2. LiChrolut EN (Merck)** **3. Isolut Env+ (IST)** **4. PLRP-S (Polymer Labs)** **5. Envchrom P (Supelco)**
Amount material (mg)	10x3 mm I.D
and size	1. 40-63 um 2. 40-63 um 3. 70-100 um 4. 20 um 5. -
Deactivation	10 ml MeOH and 10 ml H2O (deionised)
Kind of sample (ml)	2-40 ml of deionised water, tap water and river water.
Breakthrough volumes (ml)	Varying the deonised volume water from 2 to 40 ml, the results obtained were different depending on the compound, and in the cas of some PAHs (naphthalene, biphenyl, acenaphthylene and chrysene) there was a significant decrease in the recoveries when the volume of sample was higher than 10 ml.
Amount eluent (ml)	?
Fraction (nr.)	One fraction per cartridge
Drying method	Not needed (necessary)--> on-line mode.
Enrichment factor	-
Compounds	naphthalene, biphenyl, acenaphthene, fluorene, acenaphthylene, phenanthrene, 2-chlorophenol, fluoranthrene, 2,4-dinitrophenol, propham, benz[a]anthracene, 2,4-dichlorophenol, chlorpropham, chrysene, 2,4-dimethylphenol, phenol, terbutylazine, dinitro-o-cresol, 4-chloro-3-methylphenol, napropamide, carbofurane, linuron, chlorbromuron, 4-nitrophenol, desethylatrazine, desisopropylatrazine, aldicarbsulphone, fenuron, desmidepham, phenmedipham, warfarin, chlortoluron, monuron, chloroxuron, metoxuron.
Recovery	The best recoveries were obtained by Isolut Env+ and ranging from 35 to 110%.
Repeatability	Repeatability (RSD %) from 2 to 15 % and reproducibility (RSD %) from 3-14%
LOD's	LOD,s (ug/l, and S/N=10) from 0.4 to 2.6
Reference	L. Toribio et al/ J Chromatogr. A 823 (1998) 163
Remarks	The recoveries of some PAHs were low, probably due to high retention on this kind of sorbent (Isolut Env+), which resulted in an increase of their detection limits.

11	SPE
Material	**1. Carbopack B 120/400 (Supelco)** **2. HYSphere-1 5um (Spark Holland)** **3. Bond Elut PPL 125 um (Varian)**
Amount material (mg) and size	10x3 mm I.D 1. 120/400 2. 5 um 3. 125 um
Deactivation	ACN and milli-Q water (pH 2.5)
Kind of sample (ml)	different volumes of Milli-Q water, tap water and river water (50-200 ml); pH 2.5.
Breakthrough volumes (ml)	Breakthrough volumes of phenol: 1. 2 ml 2. 22 ml 3. 14 ml For all compounds: HYSphere-1 gives better recoveries varying volumes from 50-200 ml.
Amount eluent (ml)	ACN
Fraction (nr.)	One fraction per cartridge
Drying method	Not needed (necessary)--> on-line mode.
Enrichment factor	
Compounds	oxamyl, methomyl, phenol, 4-nitrophenol, 2,4-dinitrophenol, bentazol, simazine, chlor-phenoxy acid, atrazine.
Recovery	The best recoveries were obtained by HYSphere-1 and gave a higher breakthrough volume for phenol for 100 ml water sample. Recoveries were between 67% for phenol and 86% for eight polar analytes.
Repeatability	?
LOD's	LOD's were between 0.03 and 0.17 ug/l.
Reference	N. Masque et al/ J. Chromatogr. A 793 (1998) 257
Remarks	Problems arising from high contents of fulvic and humic acids were solved by adding 10% Na2SO3 solution to the sample

12	SPE
Material	**1. SDB-1 PS-DVB (Baker)** **2. SDB-1 (Baker)** **3. PLRP-S (Polymer Labs)**
Amount material (mg) and size	8x2 mm I.D 1. 200 mg, 43-123 um 2. - 3. -
Deactivation	5 ml methanol + 10 ml HPLC-grade water (pH 3)
Kind of sample (ml)	1-100 ml-1 L LC-grade water, 100 ml-1 L tap water and 100-200 ml river water (all filtred through cellulose ester filters (HA type, diameter 47 mm, pore size 0.45 um).
Breakthrough volumes (ml)	Breakthrough volumes are higher than 100 ml for each pesticides when the sample is at Ph 2 using 8x2 mm I.D. SDB-1 column, which, in general, gave the best recoveries.
Amount eluent (ml)	2 ml MeOH:ACN (50:50)
Fraction (nr.)	One fraction per cartridge
Drying method	In the off-line mode, the extracts were evaporated to dryness at 30C with a gentle stream of N2. The dry extracts were desolved in MeOH -0.005 M phosphate buffer (pH 7) (25:85, v/v). The test of volatility was performed by directly spiking the desorption solution with 50-100 ng of each analyte, then evaporating it to dryness and reconstituting the dry extract in the mixture as above. No loss of analytes was observed.
Enrichment factor	50
Compounds	Log Kow range: -0.5 to 1.7 oxamyl, methomyl, DIA (metabolite of atrazine), monocrotophos, fenuron, metamitron, DEA (metabolite of atrazine), chloridazon, carbendazim, aldicarb, aminocarb, metribuzin, methoxuron
Recovery	The best recoveries were obtained by SDB-1 sorbent and ranging from 75 and 105% for all the three types of water samples (pH 2)
Repeatability	< 10 % (RSD)
LOD's	LOD's were between 0.1 and 0.5 ug/l depending on the analytes.
Reference	S. Guenu, M-C. Hennion/ J. Chromatogr. A 737 (1996) 15
Remarks	The effect of the acidification of the samples was examined because when using off-line cartridges packed with 200 mg of the SDB-1 sorbent, arecovery of 100% was measured when 500 ml of water at pH 7 was percolated. The advantage was that humic materials were not co-extracted at pH 7 and the amount of interfering substances in surface water was low.

13	SPE
Material	**1. LiChrolut EN (Merck)** **2. GCB (Alltech)**
Amount material (mg) and size	1. 200 mg 2. 300 mg
Deactivation	10 ml acetone, 3 ml MeOH and 3 ml water (pH 2)
Kind of sample (ml)	50 ml Milli-Q water, 50 ml effluent and 50-400 ml waste water (pH 2)
Breakthrough volumes (ml)	Breakthrough volumes > 400 ml for all phenols using LiChrolut EN
Amount eluent (ml)	3x2.5 ml of ACN:MeOH (50:50)
Fraction (nr.)	One fraction per cartridge
Drying method	The combined eluate was basified with 150 ul of a o.5 mol/l methanolic solution of TMAOH to minimise evaporation losses of volatile analytes end was concentrated to 1 ml under gentle stream of N2.
Enrichment factor	
Compounds	phenol, p-cresol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 4-nitrophenol, 2,4-dinitrophenol, pentachlorophenol.
Recovery	The best recoveries were obtained with LiChrolut EN and ranging between 80 and 110%
Repeatability	< 12 % (RSD)
LOD's	2-10 ppb (HPLC-UV), 0.8-60 ppb (GC-MS, EI mode) and 0.02-0.6 ppb (GC-MS, NICI mode)
Reference	J. Cheung, R.J. Wells/ J. Chromatogr. A 771 (1997) 203
Remarks	LiChrolut EN cartridges can be re-used to preconcentrate water samples without any problems. The recoveries of phenols from effluent samples remained relatively unchanged after three repeated extraction.

14	SPE
Material	**1. SDB-1 (Baker)** **2. C18 Empore 3M disks (Baker)** **3. SDB Empore 3M disks (Baker)** **4. PLRP-S (Polymer Labs)** **5. PRP-1 (Hamilton**)
Amount material (mg)	1. 3 ml, 200 mg
and size	2. 450 mg, 11 um
	3. 450 mg, 6.8 um
	4. 10x2 mm ID, 15-25 um), 20-80 mg
	5. 100x4.6 mm ID, 10 um), 20-80 mg
Deactivation	5 ml MeOH, 10 ml
	LC-grade water
Kind of sample (ml)	500-1000 ml LC-grade water, tap water and
	river water
Breakthrough volumes (ml)	> 500 ml
Amount eluent (ml)	4 ml MeOH
Fraction (nr.)	One fraction per cartridge
Drying method	Evaporation under a stream of nitrogen: After the desorption with MeOH, 50 ul of a mixture containing MeOH and ammonia (4:1) were added before evaporation step. Under these basic conditions, acidic compounds are ionised and therefore cannot be volatilised during the evaporation step.
Enrichment factor	> 200
Compounds	Log Kow range: -0.5 to 4 oxamyl, DIA (metabolite of atrazine), DEA (metabolite of atrazine), chloridazon, carbendazim, aldicarb, methoxuron, si-mazine, 2-chlorophenol, dicamba, cyanazine, bentazone, atrazine, carbaryl, isoproturon, ioxynil, MCPP, difenoxuron, 2,4-DB, 2,4,5-T, metolachlor, dinoterb.
Recovery	The best recoveries were obtained with SDB-1 cartridges, ranging between 80-105% (pH 7)
Repeatability	?
LOD's	0.1 ug/l (river water)
Reference	V. Pichon et al/ J Chromatogr. A 737 (1996) 25
Remarks	At pH 7, humic and fulvic acids are not co-extracted using SDB-1. At pH 3, these two acids are co-extracted and interfere the more polar analytes.

15	SPE
Material	**Lichrolut EN (Merck)**
Amount material (mg) and size	250 mg, 6 ml
Deactivation	10 ml ACN, 10 ml MeOH and 10 ml distilled, dionised water (pH <2)
Kind of sample (ml)	1000 ml drinking water and other water samples
Breakthrough volumes (ml)	> 1000 ml
Amount eluent (ml)	2x3 ml ACN
Fraction (nr.)	One fraction per cartridge
Drying method	The eluate was reduced to 1 ml under gentle stream of N2
Enrichment factor	> 500
Compounds	phenol, m-cresol, o-cresol, 2,4-dimethylphenol, 2-chlorophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2-nitrophenol, 2,3,4,6- tetrachlorophenol, 4-nitrophenol, pentachlorophenol, 2,4- dinitrophenol, 4,6-dinitro-ocresol
Recovery	between 70 and 127%
Repeatability	< 20%
LOD's	LOQ's (0.015-0.075 ug/l and LOD's (0.005-0.025 ug/l)
Reference	T. Heberer, H.-J. Stan/ Analitica Chimica Acta 341 (1997) 21
Remarks	Applying SPE with LiChrolut EN adsorbent, phenols can be detected at the ng/l level in environmental sam- ples independent of the origine of the sample and its matrix load.

16	SPE
Material	**1.Sep-Pak silica (Waters)** **2. PGC (Shandon)**
Amount material (mg) and size	1. 650 mg 2. 190 mg
Deactivation	1. - 2. 2 ml MeOH, 2 ml H2O, 2ml MeOH and 2 ml H2O
Kind of sample (ml)	5-250 ml distilled water, tap water and river water
Breakthrough volumes (ml)	1. > 250 ml for the present compounds 2. 50-250 ml depending on the components
Amount eluent (ml)	1. 2 ml of 8% MeOH in 6.0 M HCl 2. 2 ml TFA:ACN (20:80)
Fraction (nr.)	One fraction per cartridge
Drying method	The eluates were evaporated at 50C almost to dryness. The residus were dissolved with 1 ml H2O
Enrichment factor	> 200
Compounds	paraquat (PQ), EQ, diquat (DQ) and difenzoquat (DF)
Recovery	between 65 and 98%
Repeatability	4-10% RSD's
LOD's	LOD's (0.2-4 ug/l)
Reference	M.C. Carneiro et al./ Analitica Chimica Acta 408 (2000) 263
Remarks	

17	SPE
Material	**1. Carbograph 1** **2. Carbograph 4**
Amount material (mg) and size	6.5x1.3 cm ID cartridges 1. 500 mg, 120x400 mesh size 2. 500 mg
Deactivation	10 ml DCM:MeOH (80:20) containing TBACl, 2 ml MeOH, 14 ml HCl-acidified water (pH 2)
Kind of sample (ml)	4000 ml drinking water and 1000 ml river water: before spiking, 0.5 g/l Na2SO3.5H2O was added to the sample to avoid oxidation of the analytes
Breakthrough volumes (ml)	> 4000 ml for tap water and > 1000 ml for river water
Amount eluent (ml)	6 ml DCM:MeOH (80:20) containing TBACl
Fraction (nr.)	One fraction per cartridge
Drying method	The extract was divided in two equal portions which were dried in a water bath at 27C under a gentle stream of N2. The first risidue was reconstituted with 200 ul of H2O/MeOH (80:20) acidified with 0.6% (v/v) TFA. The second extract with 150 ul of H2O/ACN (80:20) basified with Na2CO3, 0.1 mol/l.
Enrichment factor	> 30 000
Compounds	phenol, 2-nitrophenol, 2-chlorophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol, 2,6-dinitro-2-methylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 4-nitrophenol, 2,4-dinitrophenol, pentachlorophenol.
Recovery	between 91 and 101 % with Carbograph 4
Repeatability	1-7% RSD's
LOD's	< 1 ng/l
Reference	A. Di Corcia et al. /J. Chromatogr. A 733 (1996) 383
Remarks	If TBACl was not added to the solvent mixture, seven evaporative losses of most of the phenols were observed on drying the extract. Apparently, the presence of TBACl in the DCM:MeOH mixture makes the analytes less volatile.

18	SPE
Material	**Lichrolut EN (Merck)**
Amount material (mg) and size	200 mg, 3 ml
Deactivation	?
Kind of sample (ml)	50 ml waste water
Breakthrough volumes (ml)	Not observed by 50 ml water sample
Amount eluent (ml)	5 ml MeOH for HPLC and/or 5 ml Ethyl acetate for GC-MS
Fraction (nr.)	One fraction per cartridge
Drying method	Evaporation by nitrogen stripping to 1 ml
Enrichment factor	50
Compounds	Log Kow: -06 - 5.16 simasine; 1,2,3,4-tetrachlorobenzene; 2-hydroxydesethylatrazine; terbutylazine; 2-phenoxyethanol; triphenyl phosphate; 2-butoxyethanol acetate; 2-methylthiobenzothiazole; dibenzyl phthalate; dibutyl phthalate; dimethyl phthalate; bis(2-ethylhexyl)phthalate; benzylbutyl phthalate; 3,5-dichloroanailine; 4-nonylphenol
Recovery	> 85
Repeatability	Not reported
LOD's	?
Reference	S. Galassi et al./ J. Chromatogr. A 889 (2000) 149
Remarks	

19	SPE
Material	**Strong anion exchange resin + RP-102 in series**
Amount material (mg) and size	500 mg, 6 ml + 1 g, 6 ml
Deactivation	60 ml of 1% acetic acid in acetone+ 40 ml of 1% acetic acid in water
Kind of sample (ml)	1 l of sample waters (pH 2.8-3)
Breakthrough volumes (ml)	For more polar analytes (1 l) (see recoveries)
Amount eluent (ml)	3x4 ml of 1% acetic acid in acetone (3 ml/min)
Fraction (nr.)	One fraction per two cartridges in series
Drying method	2 times evaporation to dryness by Zymark solvent reduction workstation and reconstituted in 0.1 ml acetonitril
Enrichment factor	10 000
Compounds	imazapyr; imazythapyr; flumetsulam; nicosulfuron; imazaquin; thifensulfuron methyl; metsulforon methyl; chlorsulforon; sulfometuron methyl; triasulfuron methyl; bensulfuron methyl; halosulfuron methyl; prosulfuron; chlorimuron ethyl; triflusulfuron methyl; primisulfuron methyl
Recovery	39-92
Repeatability	14-26% (SD's)
LOD's	< 10 ng/l level
Reference	E.T Furlong et al./ The Science of the Total environment 248 (2000) 135
Remarks	

20	SSPE
Material	**Layered column comprissing of C2 (5 g) on Isolut ENV+ (2 g) layer**
Amount material (mg) and size	C2 (5 g) + Isolut ENV+ (2 g)
Deactivation	?
Kind of sample (ml)	20 l surface water
Breakthrough volumes (ml)	Not observed
Amount eluent (ml)	sequential elution with 5 ml MeOH and 5 ml DCM
Fraction (nr.)	One fraction
Drying method	Evaporation under nitrogen (40C, for 2 h) to 1 ml MeOH
Enrichment factor	20 000
Compounds	Log Kow: 0.43 - 5.12 diethylnitrosamine; dipropylnitrosamine; desmetryn; diazinon; pentachlorophenol; pirimephosmethyl; phenanthrene; carbophenothion; nonylphenol
Recovery	?
Repeatability	?
LOD's	?
Reference	K.V. Thomas et al./ Marine Pollution Bulletin 38 (1999) 925
Remarks	

21	GPC + SPE
Material	**TSK gel HW 40s + Envi Chrom P + LiChrolut EN**
Amount material (mg) and size	120 x 5 cm + 250 mg + 200 mg
Deactivation	MeOH + phosphate-buffer
Kind of sample (ml)	200 ml water samples
Breakthrough volumes (ml)	?
Amount eluent (ml)	Sequential elution with 3 ml MeOH/H2O (1:1) and 3 ml MeOH
Fraction (nr.)	GPC (5 fractions) and SPE one fraction per two cartridges
Drying method	Evaporation by Speed-Vac concentrator to o.1 ml
Enrichment factor	2000
Compounds	-
Recovery	-
Repeatability	-
LOD's	-
Reference	N. Klinkow et al./ Wat. Res. 32 (1998) 2583
Remarks	This goal of this work was to develop a separation/fractionation scheme of organic compounds according to their molecular size (GPC) and their polarity (SPE). The method was combined with luminescence inhibition test to measure the toxicity of the fractions.

22	SPE
Material	**Bakerbond Speedisk divinylbenzene (DVB) disk C18-silica disks (Bakerbond Speedisk C18)**
Amount material (mg) and size	300 mg DVB polymers (50-mm diameter and 0.5-mm high bed) + 750 mg of C18-silica (50-mm diameter and 1-mm high bed)
Deactivation	10 ml acetoniltrile, 10 ml MeOH and 10 ml of LC-grade water
Kind of sample (ml)	250–1000 ml of surface water and drinking water samples (200 ml/min flow rate). For C18 addition of 10% of MeOH to the sample before preconcentration
Breakthrough volumes (ml)	Not observed
Amount eluent (ml)	DVB disks: 9 ml acetonitrile C18 disks: 10 ml DCM:MeOH (4:1, v/v)
Fraction (nr.)	1
Drying method	After addition of 120-µl volume of MeOH:ammonia (4:1, v/v), evaporation was commenced at 40ºC using a rotary evaporator to 1 ml and then further evaporated under a gentle stream of nitrogen to a final volume of about 100 µl.
Enrichment factor	10 000
Compounds	Alachlor; Aldicarb; Aminotriazol; Atrazine; Chlorpyrifos; Dinoterb; Diuron; Endosulfan á; Endosulfan â; Fenpropimorph; Fluzilazole; Ioxynil; Isoproturon; Lindane; Linuron; Oxydemeton-methyl; Simasine; Terbuthylazine; Trifluraline; Triallate.
Recovery	77–101%
Repeatability	3–12%
LOD's	0.01–0.05 µl/l in dinking or ground water, whereas these detection limits are still in the low 0.1µl/l levels in 1000 ml of dirty surface water samples.
Reference	V. Pichon et al./J. Chromatogr. A 795 (1998) 83
Remarks	Using the two SPE procedures, it is possible to extract any compound, either polar or non-polar (Log Kow 1–6 range). Addition of 10% of MeOH to the sample was necessary to make extraction and desorption of apolar compound efficiently. Only very polar analytes may still be difficult to extract with these sorbents. In this case, a graphitized carbon is recommended.

23	SPE
Material	**Sep-Pak Plus C18; tC18; C8; tC2; CN; Diol; NH2; and PS-1 (all from waters)**
Amount material (mg) and size	?
Deactivation	5 ml MeOH and 20 ml of distilled water
Kind of sample (ml)	Distilled water, tap water, ground water and river water (50–1000 ml volumes)
Breakthrough volumes (ml)	Only for Asulam (Log Kow –0.5), breakthrough volume of 500 ml. For the rest there is no problem even for 1000 ml if the good condition are used.
Amount eluent (ml)	1–5 ml of 50% v/v MeOH–water or 1–5 ml of MeOH depending on the polarity of the analytes.
Fraction (nr.)	1
Drying method	-
Enrichment factor	Up to 1000
Compounds	**Log Kow –05–5.38 range** Butan-2-one; Acetanilidine; Benzonitrile; Acetophenone; Nitrobenzene; Benzene; Methyl benzoate; Fluorobenzene; Ethyl benzoate; Toluene; Chlorobenzene; Brobobenzene; Ethylbenzene; Iodobenzene; p-Xylene; o-Xylene; 1,4-Dichlorobenzene; Phenyl benzoate; Naphthalene; Propylbenzene; Benzyl benzoate; 1,2,4-Trichlorobenzene; Phenanthrene; Butylbenzene; Fluoranthene; 1,2,3,5-Tetrachlorobenzene; Asulam; Metolcarb; Propoxur; Carbaryl; Xylylcarb; Macbal; Cyanophos; Dichlobinil; Phosmet; Fenobcarb; p-Dichlorobenzene; o-Dichlorobenzene; Fenitrothion; Mecoprop; Chlorothalonil; Fenthion; Phenthoate; Isixathion; Quintozene; Tolclophos-methyl; Dichlofenthion.
Recovery	Depend on the polarity of the analytes, sorbent, sample volume and elution solvent. For polar analytes the best recoveries were obtained with PS-1 (85-100%). For non-polar ones, C18 gives the best results (85-100%).
Repeatability	-
LOD's	-
Reference	M. Nakamura et al./ The Analyst, 121 (1996) 469
Remarks	Recoveries of the studied compounds were related to their Log Kow. Apolar compounds were easily sorbed, but were difficult to elute. The extent of elution could be improved by adding methanol to the sample solution.

24	SPE
Material	**Bakerbond C18 or Polar Plus C18 (Baker or Supelco)** **Apolar PS-DVB; Isolute ENV+ (IST)** **Empore disks: C18 and PS-DVB (Baker)**
Amount material (mg) and size	500 mg C18 and 200 mg PS-DVB or Isolute ENV+
Deactivation	Cartridges: 5 ml MeOH + 10 ml LC-grade water Disks: 10 ml MeOH:ACN (1:1) + 10 ml MeOH + 20 ml LC-grade water
Kind of sample (ml)	500–1000 ml water samples (10 ml/min percolation flow rate for cartridges and 25 ml/min for disks).
Breakthrough volumes (ml)	Depend on Log Kw values an sorbent
Amount eluent (ml)	Cartridges: C18 (4 ml MeOH) and PS-DVB (2 ml MeOH:ACN (1:1)). Disks: C18 (2x4 ml MeOH:ACN (1:1)) and PS-DVB (2x6 ml MeOH:ACN (1:1))
Fraction (nr.)	1
Drying method	Under gentle stream of nitrogen
Enrichment factor	> 1000
Compounds	**Log Kow –0.5– 3** Oxamyl; Methomyl; Aldicarb; Carbendazim; Carbofuran; Aminocarb; Carbaryl Captan; Methiocarb; Fenuron; Metoxuron; Monuron; Monolinuron; Chlortoloron; Isoproturon; Diuron; Desethylatrazine; Hydroxyatrazine; Simazine; Simetryne; Prometon; Propazine; Terbutylazine; Metamitron; Metribuzine; Hexazinone.
Recovery	The best recoveries were obtained with Isolute ENV+ and PGC (carbon). The recoveries ranging fron 85% to 100%.
Repeatability	-
LOD's	-
Reference	M.-C. Hennion et al./J. Chromatogr. A 823 (1998) 147
Remarks	The selection of the sorbents for the extraction of polar analytes is based on Log Kw values.

25	SPE
Material	**Carbopack B 120/400 (Supelco)** **ENVI Chrom P (Supelco)**
Amount material (mg) and size	500 mg of Carbopack B and 500 mg ENVI Chrom P (80–160 µm particle size)
Deactivation	10 ml of MeOH (ENVI Chrom P) or DCM (Carbopack B) + 10 ml water and 2 ml of a 5 mM tetrabutylamonium bromide (TBA) solution.
Kind of sample (ml)	500 ml of Milli-Q-purified water, tap water samples (pH 9.0)
Breakthrough volumes (ml)	> 1000 ml except phenol.
Amount eluent (ml)	Carbopack B: 5 ml DCM + 1% of acetic acid ENVI Chrom P: 5 ml MeOH + 1% of acetic acid
Fraction (nr.)	1
Drying method	Under vacuum with rotary evaporator to 1 ml.
Enrichment factor	500
Compounds	Phenol; 4-Nitrophenol; 2,4-Dinitrophenol; 2-Chlorophenol; 2-Nitrophenol; 2,6-Dimethylphenol; 2,4-Dimethylphenol; 2-Methyl-4,6-dinitrophenol; 4-Chloro-3-methylphenol; 2,4-Dichlorophenol, 2,4,6-trimethylphenol; 2,4,6-Trichlorophenol; pentachlorophenol.
Recovery	All higher than 90% except for phenol (54%, breakthrough volume of 1000 ml).
Repeatability	< 10%
LOD's	Direct injection: 30–100 µg/l Carbon cartridge: 2–6 µg/l Polymere cartridge: 60–100 ng/l
Reference	E. Pocurull et al./J. Chromatogr. A 719 (1996) 105
Remarks	To increase the retention of the most polar compounds, mainly phenol, TBA was used as an ion-pair reagent in both sorbents, an increase in the breakthrough volumes, especially that correspond to phenol, which was the most polar compound studied.

26	SPE
Material	**Isolute ENV+** **PLRP-S** **LiChrolut** **PGC (carbon)**
Amount material (mg) and size	10 x 0.2 mm I.D. stainless-steel precolumns
Deactivation	5 ml MeOH + 1 ml water (pH 3) at 1 ml/min
Kind of sample (ml)	10–200 ml water samples (pH 2.5) at 4 ml/min
Breakthrough volumes (ml)	Isolut ENV+ and Lichrolut have the highest breakthrough volumes. By PGC there is desorption problem, so the majority of the analytes cannot be analyzed. PGC gave good results only for aminophenols.
Amount eluent (ml)	Backflush mode desorption
Fraction (nr.)	1
Drying method	- (on-line mode)
Enrichment factor	> 500
Compounds	Catechol; Phenol; 4-Nitrophenol; 2,4-Dinitrophenol; 2-Chlorophenol; 3-Chlorophenol; 4-Chlorophenol; 2-Nitrophenol; 2,6-Dimethylphenol; 2,4-Dimethylphenol; 2-Methyl-4,6-dinitrophenol; 4-Chloro-3-methylphenol; 2,4-Dichlorophenol, 2,4,6-trimethylphenol; 2,3,5-Trichlorophenol; 2,3,4-Trichlorophenol; 3,4,5-Trichlorophenol; pentachlorophenol; 2-Amino-4-chlorophenol; 4-Chloro-2-aminophenol.
Recovery	Good recoveries were obtained for all sorbents for 100 ml sample, except for phenol, catechol and 4-chloro-2-aminophenol (V = 50 ml). (55–105%).
Repeatability	< 12%
LOD's	Ground water: 0.01–7 µg/l River water: 0.02–14 µg/l
Reference	D. Puig et al./ J. Chromatogr. A 733 (1996) 371
Remarks	A matrix effect study showed that acidification of the sample is necessary to avoid binding of some analytes to the humic substances and to prevent their partial deprotonation.

27	SPE
Material	**Carbograph 1 (GCB)** **Carbograph 4 (GCB)** **Carbograph 5 (GCB)** **LiChrolut (PS-DVB) (Merck)** **Envi-Chrom P (PS-DVB) (Supelco)**
Amount material (mg) and size	GCB's: 37–150 µm particle size. LiChrolut: 1200 m^2/g surface area and 40–120 µm particle size range. Envi-Chrom P: 900 m^2/g surface area and 80–160 µm particle size range.
Deactivation	-
Kind of sample (ml)	4000 ml Tap water and 500–1000 ml river water samples
Breakthrough volumes (ml)	No breakthrough observed.
Amount eluent (ml)	GCB's: 1 ml MeOH + 6 ml of DCM:MeOH (4:1) + 6 ml of DCM:MeOH (4:1) containing 10 mmol/l tetrabutylamonium chloride (TBACl) or trifluoroacetic acid (TFA). LiChrolut and Envichrom P: 2x5 ml MeOH:ACN (1:1)
Fraction (nr.)	1
Drying method	Extracts were dried in a water bath at 30ºC by allowing a gentle stream of nitrogen. Before concentrating extracts, addition of KOH was necessary to avoid evaporative losses of the most volatile phenols.
Enrichment factor	2000–8000
Compounds	Omethoate; Aldicarb sulfone; Butocarboxim sulfoxide; Aldicarb sulfoxide; Butoxycarboxim; Oxamyl; Methomyl; Monocrotophos; Atrazine, desethyl; Metamitron; Metribuzin; Chloridazon; 2,4-Dinitroph; 4,6-Dinitro-2-methylphenol; Pentachlorophenol; Linuron; Aldicarb; Dichlorprop; 2,4,5-Thrichlorophenoxy acetic acid; Ioxynil; 2,4-Dichlorophenoxy acetic acid; 2,4-Dichlorophenoxy butyric acid; Mecoprop; 4-Nitrophenol; 2-Chlorophenol; 2-Nitrophenol; Pichloram; Chloramben; Dicamba; Bentazone; Phenol; Sulfophenyl-4-propionic acid; Sulphonyl-4-butyric acid; Sulfophenyl-4-valeric acid; Nonylphenoxyacetic acid; Dimethoate; Monuron; Simasine; Propoxur; Isoproturon.
Recovery	Carbograph 4 gave the best results with less problems compared to other sorbent and especially Carbograph 5. Recoveries ranging from 85% to 100% including very polar analytes.
Repeatability	-
LOD's	Very low ng/l level
Reference	C. Crescenzi et al./ J. Chromatogr. A 733 (1996) 41
Remarks	When analyzing analyte mixtures both acidic and neutral in nature, a differential elution scheme was adopted. Base/neutral compounds were firstly eluted by 1 ml of methanol followed by 6 ml of CH2Cl2:Ch3OH (80:20,v/v). Acidic compounds were successively eluted and collected separately by passing 6 ml of the solvent mixture having the same composition as that mentioned above, but containing 10 mmol/l TBACl.

28	SPE
Material	**Carbograph 1 (Alltech)** **LiChrolut-EN (Merck)** **PS-DVB empore disks**
Amount material (mg) and size	Carbograph 1: 37–150 µm particle size LiChrolut-EN: 1200 m^2/g surface area and 40–120 µm particle size PS-DVB empore disks: 47 mm diameter and 0.5 mm thick and 500 mg sorbent in it.
Deactivation	Carbograph 1: 7 ml of DCM:MeOH (4:1) acidified with 50 mmol/l formic acid + 5 ml MeOH + 20 ml of LC-grade water acidified with 10 mmol/l HCl. LiChrolut-EN: 5 ml of MeOH:ACN (1:1) + 5 ml of LC-grade water
Kind of sample (ml)	1000–2000 ml water samples.
Breakthrough volumes (ml)	Occur when polymer sorbent was used by the above mentioned sample volumes.
Amount eluent (ml)	Carbograph 1: 8 ml DCM:MeOH (4:1) acidified with 50 mmol/l formic acid. Lichrolut EN: 2x3 ml of MeOH:ACN (1:1). PS-DVB Empore disks: 3x3 ml of MeOH:ACN (1:1).
Fraction (nr.)	1
Drying method	Under gentle nitrogen stream by 40ºC after adding 0.4 mol/l of tetrabutylamonium fluoride (TBAF) in methanol.
Enrichment factor	2000–4000
Compounds	Fluazifop; Clodinafop; Quizalofop; Fenoxaprop; Haloxyfop; Diclofop.
Recovery	Carbograph 1 was superior: Recoveries 90–98% range
Repeatability	-
LOD's	7-20 ng/l for drinking water and 16–36 ng/l for spring water.
Reference	A. Lagana et al./ J Chromatogr. A 796 (1998) 309
Remarks	A good extraction efficiency for the six herbicides from acidified water was obtained with both LiChrolut-EN cartridge and PS-DVB Empore disk.

Annex 2 List of responsive compounds

Seq. nr	Compound name	S9	RIZA	CAS.Reg.No	Smiles Epiwin
1	1,1-Dichloroacetone		yes	513-88-2	ClC(Cl)C(=O)C
2	1,2:3,4-Dibenzoanthracene			215-58-7	c5ccc4cc3c1ccccc1c2ccccc2c3cc4c5
3	1,2:3,4-Diepoxybutane			298-18-0	C1OC1C2CO2
4	1,2-Diaminobenzene(+ S9)	yes		95-54-5	Nc(c(N)ccc1)c1
5	1,2-Dimethylhydrazine			540-73-8	CNNC
6	1,2-Epoxybutan		yes	106-88-7	O(C1CC)C1
7	1,3-Dinitropyrene			75321-20-9	O=N(=O)c4c2c3c(c(c4)N(=O)=O)ccc1c3c(ccc1)cc2
8	1,3-Dioxane			505-22-6	O1COCCC1
9	1,5-Dinitronaphthalene			605-71-0	c1cc2c(N(=O)=O)cccc2c(N(=O)=O)c1
10	1,6-Dinitropyrene			42397-64-8	O=N(=O)c1ccc2ccc3c(ccc4ccc1c2c34)N(=O)=O
11	1,8-Dinitropyrene			42397-65-9	O=N(=O)c1ccc2ccc3ccc(N(=O)=O)c4ccc1c2c34
12	1-Bromo pentane			110-53-2	BrCCCCC
13	1-Chloro-2,4-dinitrobenzene		yes	97-00-7	O=N(=O)c(ccc(c1N(=O)=O)Cl)c1
14	1-Naphthylamine		yes	134-32-7	c(c(c(N)cc1)ccc2)(c2)c1
15	1-Nitronaphthalene		yes	86-57-7	O=N(=O)c(c(c(ccc1)cc2)c1)c2
16	1-Nitropyrere			5522-43-0	O=N(=O)c(c(c(c(c(c1)ccc2)c2cc3)c3c4)c1)c4
17	2,4,7-Trinitro-9-fluorene			34263-36-0	O=[N+]([O-])C3=CC1=C(C=C3)C2=C(C=C([N+]([O-])=O)C=C2[N+]([O-])=O)C1
18	2,4-Diaminotoluene (+ S9)	yes		95-80-7	Nc(c(ccc1N)C)c1
19	2,4-Dimethyl-1,3-dioxane			766-20-1	O(CCC1C)C(O1)C
20	2,4-Dinitrotoluene		yes	121-14-2	O=N(=O)c(ccc(c1N(=O)=O)C)c1
21	2,6-Diaminotoluene (+ S9)	yes		823-40-5	Nc(c(c(N)cc1)C)c1
22	2,7-Dinitro-8-fluorenone			31551-45-8	O=C(c(c(c1ccc(N(=O)=O)c2)ccc3N(=O)=O)c3)c12
23	2-Acetylaminofluorene (+S9)	yes	yes	53-96-3	O=C(Nc(ccc(c1Cc2cccc3)c23)c1)C
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	yes		77094-11-2	CC2=CC1=NC=CC=C1C3=C2N(C)C(N)=N3

25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9)	yes		76180-96-6	Cc3cc1c(ccc2ncnc12)nc3N
26	2-Amino-3-methyl-9H-pyrido(2,3-b)indole; MeA alpha C (+ S9)	yes		68006-83-7	Cc1cc2c(nc1N)nc3ccccc23
27	2-Amino-6-methyidipyrido(1,2-a:3',2'd)imidazole (Glu-P-l;+ S9)	yes		67730-11-4	CC1=CC=Cn2c1nc3ccc(N)nc23
28	2-Amino-9H-pyrido(2,3-b)indole (+S9)(A alpha C)	yes		26148-68-5	Nc1ccc2c(nc3c2cccc3)n1
29	2-Aminoanthracene(+ S9)	yes		613-13-8	Nc3ccc2cc1ccccc1cc2c3
30	2-Amino-dipyrido(1,2-a:3',2'-d)imidazole-2-amine (Glu-P-2; + S9)	yes		67730-1 0-3	Nc3ccc2nc1C=CC=Cn1c2n3
31	2-Aminofiuorene(+S9)	yes	yes	153-78-6	Nc3ccc1c(Cc2ccccc12)c3
32	2-Amino-l-methyl-6-phenylimidazo[4,5-b]pyridine (PhlP)			105650-23-5	CN1C(N)=NC2=C1C=C(C3=CC=CC=C3)C=N2
33	2-Nitrofluorene			607-57-8	O=N(=O)c(ccc(c1Cc2cccc3)c23)c1
34	2-Nitronaphthalene			581-89-5	O=N(=O)c2ccc1ccccc1c2
35	2-Nitro-p-phenylenediamine			5307-14-2	O=N(=O)c(c(N)ccc1N)c1
36	3,7-Dinitrofluoranthene			105735-71-5	O=N(=O)c1ccc2c4cccc(c4c3c2c1ccc3)N(=O)=O
37	3,9-0initrofluoranthene			22506-53-2	[O-][N+](C2=CC=C3C1=C(C4=C3C=C([N+]([O-])=O)C=C4)C=CC=C12)=O
38	3-Amino-1,4-dimethyl.SH-pyrido[3,4-b]lindole; Trp-P-1 (+ S9)	yes		62450-06-0	Cc2nc(N)c(C)c3c1ccccc1nc23
39	3-Amino-l-methyl-5H-pyrido[4,3-b]indole; Trp-P 2 (+ S9)	yes		62450-07-1	CC1=NC(N)=CC2=C1C(C=CC=C3)=C3N2
40	3-Methoxy-4-aminoazobenzene(+S9)	yes		3544-23-8	COc1c(N)ccc(N=Nc2ccccc2)c1
41	3-Methylcholanthrene (+ S9)	yes	yes	56-49-5	c(c(ccc1C)cc(c2ccc3cccc4)c34)(c1CC5)c25
42	3-Nitrofluoranthene			892-21-7	O=N(=O)c2ccc3c1ccccc1c4cccc2c34
43	4,4'-Dinitrobiphenyl			1528-74-1	O=N(=O)c(ccc(c(ccc(N(=O)=O)c1)c1)c2)c2
44	4-Aminobiphenyl		yes	92-67-1	Nc(ccc(c(cccc1)c1)c2)c2
45	4-Methyl-1,3-dioxane			1120-97-4	O1COC(C)CC1
46	4-Nitro-o-phenylenediamine			99-56-9	O=N(=O)c(ccc(N)c1N)c1
47	4-Nitroquinoline-N-oxide		yes	56-57-5	O=N(=O)c1ccn(=O)c2ccccc12

48	4-Nitrosodiphenylamine			156-10-5	O=Nc(ccc(Nc(cccc1)c1)c2)c2
49	4-Nitroso-N,N-dimethylaniline			138-89-6	O=Nc(ccc(N(C)C)c1)c1
50	5-Butyrolactone			36536-46-6	O1C(=O)CC1C
51	5-Fluorouracil			51-21-8	N1C(=O)NC(=O)C(F)=C1
52	5-MOP (+ UV)			484-20-8	O(C=C1)c(cc(OC(=O)C=2)c3C2)c1c3OC
53	5-Nitro-2-furaldehyde			698-63-5	N(=O)(=O)c1ccc(C(=O))o1
54	5-Nitro-2-furylacrylacid			710-25-8	O1C(N(=O)=O)=CC=C1C=CC(=O)N
55	5-Nitroacenaphthene			602-87-9	O=N(=O)c(c(c(c(cc1)CC2)c2c3)c1)c3
56	6-Aminochrysene(+ S9)	yes		2642-98-0	Nc3cc2c1ccccc1ccc2c4ccccc34
57	6-chloro-9-(3-(2-chloroethylamino)propyl)amino-2-rnethoxyacridine*2HCl(ICR-191)			17070-45-0	COC3=CC=C2N=C1C=C(Cl)C=CC1=C(NCCCNCCCl)C2=C3
58	6-Nitrochrysene			2-89-6	CC1CC2OC(=O)C(=C)C2C(OC(=O)CCCC(=O)OC3C4C(CC(C)C5C=CC(=O)C53C)OC(=O)C4=C)C6(C)C1C=CC6=O
59	7,12-Dimethylbanzanthracene (+ S9)	yes	yes	57-97-6	c(c(c(c(c1)ccc2)c2)c(c(c3ccc4)c4)C)(c3C)c1
60	8-MOP (+ UV)			298-81-7	O=C1C=Cc2cc3ccoc3c(OC)c2O1
61	8-Proplotactone			57-57-8	O=C(OC1)C1
62	9 Aminoacridine			90-45-9	Nc2c1ccccc1nc3ccccc23
63	Acridine Orange			494-38-2	CN(c1cc2c(cc1)cc3c(n2)cc(cc3)N(C)C)C
64	Acrinol(+S9)	yes		1837-57-6	NC1=C(C=C(OCC)C=C3)C3=NC2=C1C=CC(N)=C2
65	Adriamycin(Adr)			25316-40-9	COC1=C(C(C(C(O)=C([C@@H](O[C@H]5CC(N)C(O)[C@H](C)O5)C[C@@](C(CO)=O)(O)C4)C4=C3O)=C3C2=O)=O)C2=CC=C1
66	Afiatoxin G1			1165-39-5	C12C=COC1Oc3cc(OC)c(C(C5)=C(C(=O)OC5)C(=O)O4)c4c23
67	Aflatoxin B1			1162-65-8	C12C=COC1Oc3cc(OC)c(C(C5)=C(C(=O)C5)C(=O)O4)c4c23
68	Amphotericin B			1397-89-3	O=CCC(CC(CCC(C(CC(CC1(OC(CC(OC2C(C(N)C(C(O2)C)O)O)C=CC=CC=CC=CC=CC=CC(C(C(C(O)C)C)O)C)C(C(C1)O)C(=O)O)O)O)O)O)O)O
69	Amsacrine			51264-14-3	COc1cc(NS(C)(=O)=O)ccc1Nc3c2ccccc2nc4ccccc34
70	Auramine			2465-27-2	CN(C)c1ccc(cc1)C(=N)c2ccc(cc2)N(C)C
71	Avarol(+ S9)	yes		55303-98-5	OC(C(C[C@]([C@H](CC[C@@]23C)C)(C2CCC=C3C)C)=C1)=CC=C1O
72	Azaserine			115-02-6	NC(COC(=O)C=N#N)C(O)=O

73	Azinphos-Methyl		yes	86-50-0	S=P(OC)(OC)SCN1N=Nc2ccccc2C1(=O)
74	Azobenzene(+ S9)	yes	yes	103-33-3	N(=Nc(cccc1)c1)c(cccc2)c2
75	Benz(a)anthracene(+ S9)	yes	yes	56-55-3	c(c(c(c(c1)ccc2)c2)cc(c3ccc4)c4)(c1)c3
76	Benzidine (+ S9)	yes	yes	92-87-5	Nc(ccc(c(ccc(N)c1)c1)c2)c2
77	Benzo(a)pyrene(+ S9)		yes	50-32-8	c(c(c(cc1)ccc2)c2cc3)(c3cc(c4ccc5)c5)c14
78	Benzylchloride		yes	100-44-7	c(cccc1)(c1)CCl
79	Bleomycin			11056-06-7	O=C(N)C[C@@H](C1=NC(N)=C(C)C(C(N[C@@]([H])([C@@](C5=CNC=N5)([H])OC4C(OC6C(O)C(OC(N)=O)C(O)C(CO)O6)C(O)C(O)C(CO)O4)C(N[C@]([C@H]([C@H](C)C(N[C@]([C@](O)(C)[H])([H])C(NCCC2=NC(C3=NC(C(NCCC(SH)(C)C)=O)=CS3)=CS2)=O)=O)O)([H])C)=O)=O)=N1)NC[C@H](N)C(N)=O
80	Bromo acetic acid			79-08-3	O=C(O)CBr
81	Bromobenzene		yes	108-86-1	c(cccc1)(c1)Br
82	Captan		yes	133-06-2	O=C(N(SC(Cl)(Cl)Cl)C(=O)C1CC=CC2)C12
83	Chloramine T			127-65-1	ClN([Na])S(=O)(=O)c1ccc(C)cc1
84	Chromium(VI)oxide			1333-82-0	[Cr](=O)(=O)=O
85	Chrysene (+ S9)	yes	yes	218-01-9	c1ccc2ccc3c4ccccc4ccc3c2c1
86	Ciprofloxacin			85721-33-1	C1CNCCN1c2cc3N(C4CC4)C=C(C(=O)O)C(=O)c3cc2F
87	cis-Platinum			15663-27-1	Cl[Pt](Cl)(N(H)(H)H)N(H)(H)H
88	Citrinin			518-75-2	CC2OC=C1C(=C(C(O)=O)C(=O)C(=C1C2C)C)O
89	Cumenehydroperoxide			80-15-9	O(O)C(c(cccc1)c1)(C)C
90	Cupferron			135-20-6	NON(N=O)c1ccccc1
91	Danthron			117-10-2	O=C(c(c(c(O)cc1)C(=O)c2c(O)ccc3)c1)c23
92	Daunomycin			23541-50-6	COC1=C(C(C(C(O)=C([C@@H](O[C@H]5CC(N)C(O)[C@H](C)O5)C[C@@](C(C)=O)(O)C4)C4=C3O)=C3C2=O)=O)C2=CC=C1
93	Daunorubicin			20830-81-3	COc4cccc5C(=O)c3c(O)c2CC(O)(CC(OC1CC(N)C(O)C(C)O1)c2c(O)c3C(=O)c45)C(C)=O
94	Dibromoacetonitrile		yes	3252-43-5	N#CC(Br)Br
95	Dichlofluanid			1085-98-9	CN(C)S(=O)(=O)N(SC(F)(Cl)Cl)c1ccccc1
96	Diethylnitrosamine (+ S9)	yes	yes	55-18-5	O=NN(CC)CC
97	Diethylsulfate (+ S9)	yes	yes	64-67-5	O=S(=O)(OCC)OCC
98	Dimethylnitrosamine (+ S9)	yes	yes	62-75-9	O=NN(C)C

99	Dimethylsulfate		yes	77-78-1	O=S(=O)(OC)OC
100	Dimethylsulfoxide			67-68-5	O=S(C)C
101	Dithianone			3347-22-6	c1ccc2C(=O)C(S3)=C(SC(C#N)=C3C#N)C(=O)c2c1
102	Doxorubicinhydrochloride			25316-40-9	
103	Ellipticine			519-23-3	c1ccc2nc3c(C)c4ccncc4c(C)c3c2c1
104	Enoxacin			74011-58-8	C1CNCCN1c2nc3N(CC)C=C(C(=O)O)C(=O)c3cc2F
105	Epichlorhydrin		yes	106-89-8	O(C1CCl)C1
106	Epoxystyrene			96-09-3	O(C1c(cccc2)c2)C1
107	Ethidium bromide (+ S9)	yes		1239-45-8	CCn3(Br)c(c1ccccc1)c2cc(N)ccc2c4ccc(N)cc34
108	Ethyl methanesulfonate (EMS)		yes	62-50-0	O=S(=O)(OCC)C
109	Ethylene dibromide		yes	106-93-4	BrCCBr
110	Folpet			133-07-3	O=C(N(SC(Cl)(Cl)Cl)C(=O)c1cccc2)c12
111	Formaldehyde			50-00-0	O=C
112	Furazolidone			67-45-8	O=C(OCC1)N1N=CC(OC(N(=O)=O)=C2)=C2
113	Furylfuramide (AF-2)			3688-53-7	NC(=O)C(=Cc1ccc(o1)N(=O)=O)c2ccco2
114	Glutaraldehyde			111 30-8	O=CCCCC=O
115	Glyoxal			4405-13-4	C12OC(O)C(O)OC1OC(O)C(O)O2
116	Harmane			486-84-0	Cc1nccc2c3ccccc3nc12
117	Hydrazine sulfate (+ S9)	yes		10034-93-2	NNOS(O)(=O)=O
118	Hydrogen peroxide			7722-84-1	OO
119	Hydroxy urea			127-07-1	NC(=O)NO
120	m-Dinitrobenzene			99-65-0	O=N(=O)c(cccc1N(=O)=O)c1
121	Methapyrilene (+ S9)	yes	yes	91-80-5	CN(C)CCN(Cc1cccs1)c2ccccn2
122	Methyl methanesulfonate (MMS)			66-27-3	O=S(=O)(OC)C
123	Methylbromide			74-83-9	BrC
124	Methylene bromide		yes	74-95-3	BrCBr
125	Methylenebromide	yes(?)	yes	74-95-3	BrCBr
126	Metronidazol			443-48-1	Cc1ncc(N(=O)=O)n1CCO
127	Mitomycin C			50-07-7	O=C1C(N)=C(C)C(=O)C2=C1C(COC(=O)N)C3(OC)C(N4)C4CN23
128	Nalidixic acid (free acid)			389-08-2	Cc1ccc2C(=O)C(C(=O)O)=CN(CC)c2n1
129	Nalidixic acid (sodium-salt)			3374-05-8	Cc1ccc2C(=O)C(C(=O)O[Na])=CN(CC)c2n1
130	N-Ethyl-N*-nitro-N-nitrosoguanidine			4245-77-6	CCN(N=O)C(=N)NN(=O)=O

131	N-Ethyl-N-nitrosourea			759-73-9	O=C(N(N=O)CC)N
132	Nifuroxazide			965-52-6	O=C(NN=Cc(oc(N(=O)(=O))c1)c1)c(ccc(O)c2)c2
133	Nitrofurantoin			67-20-9	O=C(N(N=CC(OC(N(=O)=O)=C1)=C1)CC2=O)N2
134	Nitrofurazone			59-87-0	O=C(N)NN=CC(OC(N(=O)=O)=C1)=C1
135	Nitrogen dioxide			10102-44-0	O=N=O
136	N-Methyl-N-nitro-N-nitrosoguanidine			70-25-7	O=N(=O)NC(=N)N(N=O)C
137	N-Methyl-N-nitrosourea			684-93-5	O=C(N(N=O)C)N
138	N-Nitrosobutylurea			869-01-2	CCCCN(N=O)C(N)=O
139	N-Nitrosocimetidine			73785-40-7	
140	N-Nitrosodiethanolamine			1116-54-7	O=NN(CCO)CCO
141	N-Nitrosodiphenyiamine		yes	86-30-6	O=NN(c(cccc1)c1)c(cccc2)c2
142	N-Nitroso-N-butyl-N-propylamine			25413-64-3	CCCCN(N=O)CCC
143	Norharmane			244-63-3	c1ccc2c(c1)nc3cnccc23
144	Nystatin			1400-61-9	O=C(O)CCCCCCCC=CC=CC=CC=CCC
145	o-Aminoazotoluene			97-56-3	N(=Nc(c(ccc1)C)c1)c(ccc(N)c2C)c2
146	Ofloxacin			82419-36-1	C1CN(C)CCN1c2c(F)cc3C(=O)C(C(=O)O)=CN4c3c2OCC4C
147	o-Nitroanisol			91-23-6	O=N(=O)c(c(OC)ccc1)c1
148	o-Tolidine		yes	119-93-7	Nc(c(cc(c(ccc(N)c1C)c1)c2)C)c2
149	Paraquat			4685-14-7	Cn1(Cl)ccc(cc1)c2ccn(Cl)(C)cc2
150	Phenobarbital			50-06-6	CCC1(C(=O)NC(=O)NC1=O)c2ccccc2
151	Phenylhydrazine			100-63-0	N(N)c(cccc1)c1
152	Pikrinic acid (+ S9)	yes		88-89-1	O=N(=O)c(cc(N(=O)=O)c(O)c1N(=O)=O)c1
153	Potassium chromate			11073-34-0	
154	Potassium dichromate (Cr207 2-)			7778-00-9	
155	p-Phenylendiamine		yes	106-50-3	Nc(ccc(N)c1)c1
156	Propane sultone			1120-71-4	O=S(=O)(OCC1)C1
157	Propylene oxide			75-56-9	O(C1C)C1
158	Pyrogallol			87-66-1	Oc(c(O)ccc1)c1O
159	Quercetin(+ S9)	yes		117-39-5	Oc1cc(O)c2C(=O)C(O)=C(c3cc(O)c(O)cc3)Oc2c1
160	Saccharin			81-07-2	O=C(NS(=O)(=O)c1cccc2)c12
161	Selene dioxide			7446-08-4	
162	Sodium nitrite(NO2-)			7632-00-0	[Na]ON=O

163	Sterigmatocystin		10048-13-2	COc4cc2OC1OC=CC1c2c5oc3cccc(O)c3C(=O)c45
164	Streptonigrin		3930-19-6	c42C(=O)C(OC)=C(N)C(=O)c4nc(c1nccc(c3c(O)c(OC)c(OC)cc3)c1N)cc2
165	Streptozotocin		18883-66-4	CN(N=O)C(=O)NC1C(O)OC(CO)C(O)C1O
166	Styrene oxide		96-09-3	O(C1c(cccc2)c2)C1
167	t-Butylhydroperoxide	yes	75-91-2	O(O)C(C)(C)C
168	Trichloroacetone	yes	918-00-3	ClC(Cl)(Cl)C(=O)C
169	Trichloronitromethane		76-06-2	O=N(=O)C(Cl)(Cl)Cl
170	Tris(2,3-dibromopropyl)phosphate		126-72-7	O=P(OCC(Br)CBr)(OCC(Br)CBr)OCC(Br)CBr

Annex 3 Predicted and measured values for Kow

Nr.	Compound	LogKow predicted	LogKow Exp	LogKow selected	References Kow exp	Remarks Kow
1	1,1-Dichloroacetone	0.2		0.2		
2	1,2:3,4-Dibenzoanthracene	6.7	6.41	6.41	Helweg,C et al. (1997a)	
3	1,2:3,4-Diepoxybutane	-0.58	-0.28	-0.28	Deneer,JW et al. (1988)	
4	1,2-Diaminobenzene(+ S9)	0.16	0.15	0.15	Hansch,C et al. (1995)	
5	1,2-Dimethylhydrazine	-0.54		-0.54		
6	1,2-Epoxybutan	0.86		0.86		
7	1,3-Dinitropyrene	4.57		4.57		
8	1,3-Dioxane	0.18		0.18		
9	1,5-Dinitronaphthalene	2.8	2.58	2.58	Debnath,AK et al. (1992)	
10	1,6-Dinitropyrene	4.57		4.57		
11	1,8-Dinitropyrene	4.57		4.57		
12	1-Bromo pentane	3.14	3.37	3.37	Hansch,C et al. (1995)	
13	1-Chloro-2,4-dinitrobenzene	2.27	2.17	2.17	Debnath,AK et al. (1991)	
14	1-Naphthylamine	2.25	2.25	2.25	Hansch,C et al. (1995)	
15	1-Nitronaphthalene	2.99	3.19	3.19	Hansch,C et al. (1995)	
16	1-Nitropyrere	4.75	5.06	5.06	BioByte (1995)	
17	2,4,7-Trinitro-9-fluorene	3.47		3.47		
18	2,4-Diaminotoluene (+ S9)	0.16	0.14	0.14	Debnath,AK et al. (1992)	
19	2,4-Dimethyl-1,3-dioxane	1.02		1.02		
20	2,4-Dinitrotoluene	2.18	1.98	1.98	Hansch,C et al. (1995)	
21	2,6-Diaminotoluene (+ S9)	0.16		0.16		
22	2,7-Dinitro-8-fluorenone	3.19	2.84	2.84	Debnath,AK & Hansch,C (1992)	
23	2-Acetylaminofluorene (+S9)	3.12		3.12		
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	2.12	1.98	1.98	Hansch,C et al. (1995)	
25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9)	1.66		1.66		

Nr.	Compound	LogKow predicted	LogKow Exp	LogKow selected	References Kow exp
26	2-Amino-3-methyl-9H-pyrido(2,3-b)indole; MeA alpha C (+ S9)	2.48	2.9	2.9	BioByte (1995)
27	2-Amino-6-methyidipyrido(1,2-a:3',2'd)imidazole (Glu-P-l;+ S9)	2.18	1.75	1.75	Hansch,C et al. (1995)
28	2-Amino-9H-pyrido(2,3-b)indole (+S9)(A alpha C)	1.93	2.6	2.6	Hansch,C et al. (1995)
29	2-Aminoanthracene(+ S9)	3.43		3.43	
30	2-Amino-dipyrido(1,2-a:3',2'-d)imidazole-2-amine (Glu-P-2; + S9)	1.64	1.38	1.38	Hansch,C et al. (1995)
31	2-Aminofiuorene(+S9)	3.1	3.14	3.14	Debnath,AK et al. (1992)
32	2-Amino-l-methyl-6-phenylimidazo[4,5-b]pyridine (PhlP)	2.16	2.23	2.23	Hansch,C et al. (1995)
33	2-Nitrofluorene	3.83	3.37	3.37	Debnath,AK & Hansch,C (1992)
34	2-Nitronaphthalene	2.99	3.24	3.24	Debnath,AK et al. (1992)
35	2-Nitro-p-phenylenediamine	0.55	0.53	0.53	Bronaugh,RL & Congdon,ER (1984)
36	3,7-Dinitrofluoranthene	4.57		4.57	
37	3,9-0initrofluoranthene	4.57		4.57	
38	3-Amino-1,4-dimethyl.SH-pyrido[3,4-b]lindole; Trp-P-1 (+ S9)	3.02	1.97	1.97	BioByte (1995)
39	3-Amino-l-methyl-5H-pyrido[4,3-b]indole; Trp-P 2 (+ S9)	2.48	1.97	1.97	Hansch,C et al. (1995)
40	3-Methoxy-4-aminoazobenzene(+S9)	3.27		3.27	
41	3-Methylcholanthrene (+ S9)	7.05	6.42	6.42	Hansch,C et al. (1995)
42	3-Nitrofluoranthene	4.75		4.75	
43	4,4'-Dinitrobiphenyl	3.39		3.39	
44	4-Aminobiphenyl	2.84	2.86	2.86	Martin-Villodre,A et al. (1986)
45	4-Methyl-1,3-dioxane	0.6		0.6	
46	4-Nitro-o-phenylenediamine	0.55	0.88	0.88	Hansch,C et al. (1995)
47	4-Nitroquinoline-N-oxide	0.82	1.09	1.09	Hansch,C et al. (1995)
48	4-Nitrosodiphenylamine	3.16		3.16	
49	4-Nitroso-N,N-dimethylaniline	2.04		2.04	
50	5-Butyrolactone	-0.38		-0.38	

Nr.	Compound	LogKow predicted	LogKow Exp	LogKow selected	References Kow exp
51	5-Fluorouracil	-0.81	-0.89	-0.89	Hansch,C et al. (1995)
52	5-MOP (+ UV)	2.14	1.93	1.93	(in press)
53	5-Nitro-2-furaldehyde	0.65	1.01	1.01	Hansch,C et al. (1995)
54	5-Nitro-2-furylacrylacid	0.01	0.65	0.65	Balaz,S et al. (1985)
55	5-Nitroacenaphthene	3.97	3.85	3.85	Debnath,AK et al. (1992)
56	6-Aminochrysene(+ S9)	4.6	4.99	4.99	Hansch,C et al. (1995)
57	6-chloro-9-(3-(2-chloroethylamino)propyl)amino-2-rnethoxyacridine*2HCl(ICR-191)	4.4		4.4	
58	6-Nitrochrysene	2.54	1.37	1.37	BioByte (1995)
59	7,12-Dimethylbanzanthracene (+ S9)	6.62	5.8	5.8	Hansch,C et al. (1995)
60	8-MOP (+ UV)	2.14	2	2	(in press)
61	8-Proplotactone	-0.8		-0.8	
62	9 Aminoacridine	2.4	2.74	2.74	Hansch,C et al. (1995)
63	Acridine Orange	3.67		3.67	
64	Acrinol(+S9)	2.06		2.06	
65	Adriamycin(Adr)	1.85	1.27	1.27	
66	Afiatoxin G1	0.5		0.5	
67	Aflatoxin B1	1.23		1.23	
68	Amphotericin B	-2.8		-2.8	
69	Amsacrine	3.89		3.89	
70	Auramine	2.98		2.98	
71	Avarol(+ S9)	7.02		7.02	
72	Azaserine	-2.36	-2	-2	Ellington,JJ & Stancil,FE (1988)
73	Azinphos-Methyl	2.53	2.75	2.75	Hansch,C et al. (1995)
74	Azobenzene(+ S9)	4.11	3.82	3.82	Hansch,C et al. (1995)
75	Benz(a)anthracene(+ S9)	5.52	5.76	5.76	Wang,L et al. (1986)
76	Benzidine (+ S9)	1.92	1.34	1.34	Hansch,C et al. (1995)
77	Benzo(a)pyrene(+ S9)	6.11	6.13	6.13	De Maagd,PG et al. (1998)

Nr.	Compound	LogKow predicted	LogKow Exp	LogKow selected	References Kow exp
78	Benzylchloride	2.79	2.3	2.3	Hansch,C et al. (1995)
79	Bleomycin	-10.1			
80	Bromo acetic acid	0.43	0.41	0.41	Hansch,C et al. (1995)
81	Bromobenzene	2.88	2.99	2.99	Hansch,C et al. (1995)
82	Captan	2.74	2.8	2.8	Tomlin,C (1997)
83	Chloramine T	-0.5		-0.5	
84	Chromium(VI)oxide				
85	Chrysene (+ S9)	5.52	5.81	5.81	De Maagd,PG et al. (1998)
86	Ciprofloxacin	0	0.28	0.28	Takacs-Novak,K et al. (1992)
87	cis-Platinum	-2.75	-2.19	-2.19	Hansch,C et al. (1995)
88	Citrinin	0.45		0.45	
89	Cumenehydroperoxide	2.16		2.16	
90	Cupferron	-1.73		-1.73	
91	Danthron	3.94		3.94	
92	Daunomycin	2.19	1.83	1.83	Sangster (1993)
93	Daunorubicin	2.19	1.83	1.83	Sangster (1993)
94	Dibromoacetonitrile	0.47		0.47	
95	Dichlofluanid	2.72	3.7	3.7	Tomlin,C (1997)
96	Diethylnitrosamine (+ S9)	0.34	0.48	0.48	Hansch,C et al. (1995)
97	Diethylsulfate (+ S9)	1.14	1.14	1.14	Hansch,C et al. (1995)
98	Dimethylnitrosamine (+ S9)	-0.64	-0.57	-0.57	Hansch,C et al. (1995)
99	Dimethylsulfate	0.16		0.16	
100	Dimethylsulfoxide	-1.22	-1.35	-1.35	Hansch,C et al. (1995)
101	Dithianone	2.98	2.84	2.84	Hansch,C et al. (1995)
102	Doxorubicinhydrochloride				
103	Ellipticine	4.47	4.8	4.8	Hansch,C et al. (1995)
104	Enoxacin	-0.21	-0.2	-0.2	Sangster (1994) (ion-correct
105	Epichlorhydrin	0.63	0.45	0.45	Deneer,JW et al. (1988)

Nr.	Compound	LogKow predicted	LogKow Exp	LogKow selected	References Kow exp
106	Epoxystyrene	1.59	1.61	1.61	Hansch,C et al. (1995)
107	Ethidium bromide (+ S9)	-0.38		-0.38	
108	Ethyl methanesulfonate (EMS)	-0.17		-0.17	
109	Ethylene dibromide	2.01	1.96	1.96	Hansch,C et al. (1995)
110	Folpet	2.84	2.85	2.85	Hansch,C et al. (1995)
111	Formaldehyde	0.35	0.35	0.35	Hansch,C et al. (1995)
112	Furazolidone	1.02	-0.04	-0.04	Debnath,AK et al. (1991)
113	Furylfuramide (AF-2)	1.14	0.15	0.15	BioByte (1995)
114	Glutaraldehyde	-0.18		-0.18	
115	Glyoxal	-2.89		-2.89	
116	Harmane	2.75	3.1	3.1	BioByte (1995)
117	Hydrazine sulfate (+ S9)	-4.05		-4.05	
118	Hydrogen peroxide	-1.57		-1.57	
119	Hydroxy urea	-1.68	-1.8	-1.8	Hansch,C et al. (1995)
120	m-Dinitrobenzene	1.63	1.49	1.49	Hansch,C et al. (1995)
121	Methapyrilene (+ S9)	2.55	2.87	2.87	Sangster (1994)
122	Methyl methanesulfonate (MMS)	-0.66		-0.66	
123	Methylbromide	1.18	1.19	1.19	Hansch,C et al. (1995)
124	Methylene bromide	1.52	1.7	1.7	Martiska,A & Bekarek,V (1990)
125	Methylenebromide	1.52	1.7	1.7	Martiska,A & Bekarek,V (1990)
126	Metronidazol	0	-0.02	-0.02	Hansch,C et al. (1995)
127	Mitomycin C	-1.18	-0.4	-0.4	Hansch,C et al. (1995)
128	Nalidixic acid (free acid)	1.64	1.59	1.59	BioByte (1995)
129	Nalidixic acid (sodium-salt)	-2.16			
130	N-Ethyl-N*-nitro-N-nitrosoguanidine	-0.43		-0.43	
131	N-Ethyl-N-nitrosourea	-0.02	0.23	0.23	Hansch,C et al. (1995)
132	Nifuroxazide	1.49		1.49	
133	Nitrofurantoin	-0.17	-0.47	-0.47	Hansch,C et al. (1995)

Nr.	Compound	LogKow predicted	LogKow Exp	LogKow selected	References Kow exp
134	Nitrofurazone	0.23	0.23	0.23	Hansch,C et al. (1995)
135	Nitrogen dioxide				
136	N-Methyl-N-nitro-N-nitrosoguanidine	-0.92		-0.92	
137	N-Methyl-N-nitrosourea	-0.52	-0.03	-0.03	Hansch,C et al. (1995)
138	N-Nitrosobutylurea	0.96	1.04	1.04	Hansch,C et al. (1995)
139	N-Nitrosocimetidine				
140	N-Nitrosodiethanolamine	-1.28		-1.28	
141	N-Nitrosodiphenyiamine	3.16	3.13	3.13	Hansch,C et al. (1995)
142	N-Nitroso-N-butyl-N-propylamine	1.82	2.09	2.09	Vera,A et al. (1992)
143	Norharmane	2.2	3.17	3.17	Biagi,GL et al. (1989)
144	Nystatin	7.08		7.08	
145	o-Aminoazotoluene	4.29		4.29	
146	Ofloxacin	-0.2	-0.39	-0.39	Hansch,C et al. (1995)
147	o-Nitroanisol	1.89	1.73	1.73	Hansch,C et al. (1995)
148	o-Tolidine	3.02	2.34	2.34	Hansch,C et al. (1995)
149	Paraquat	-2.71		-2.71	
150	Phenobarbital	1.33	1.47	1.47	Hansch,C et al. (1995)
151	Phenylhydrazine	0.79	1.25	1.25	Hansch,C et al. (1995)
152	Pikrinic acid (+ S9)	1.54	1.33	1.33	Sangster (1994)
153	Potassium chromate				
154	Potassium dichromate (Cr207 2-)				
155	p-Phenylendiamine	-0.39	-0.3	-0.3	Hansch,C et al. (1995)
156	Propane sultone	-0.28		-0.28	
157	Propylene oxide	0.37	0.03	0.03	Hansch,C et al. (1995)
158	Pyrogallol	0.97		0.97	
159	Quercetin(+ S9)	1.48		1.48	
160	Saccharin	0.45	0.91	0.91	Hansch,C et al. (1995)
161	Selene dioxide				

Nr.	Compound	LogKow predicted	LogKow Exp	LogKow selected	References Kow exp
162	Sodium nitrite(NO2-)				
163	Sterigmatocystin	3.81		3.81	
164	Streptonigrin	-0.56		-0.56	
165	Streptozotocin	-1.61	-1.45	-1.45	Hansch,C et al. (1995)
166	Styrene oxide	1.59	1.61	1.61	Hansch,C et al. (1995)
167	t-Butylhydroperoxide	0.94		0.94	
168	Trichloroacetone	1.12		1.12	
169	Trichloronitromethane	1.32	2.09	2.09	Hansch,C et al. (1995)
170	Tris(2,3-dibromopropyl)phosphate	4.19	4.29	4.29	Sangster (1994) (avg)

References experimentally determined K_{ow} values as cited in Meylan and Howard (1999c)

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Annex 4 Predicted and experimentally determined Henry Law constants

Nr.	Compound	CAS.Reg.No.	H bond Pa.m3/Mol	H group	H exp	H selected	LogH selected	Ref HLC
1	1,1-Dichloroacetone	513-88-2	6.03E-01			6.03E-01	-0.22	
2	1,2:3,4-Dibenzoanthracene	215-58-7	4.80E-02	1.21E-02		4.80E-02	-1.32	
3	1,2:3,4-Diepoxybutane	298-18-0	8.08E-02	3.47E-03		8.08E-02	-1.09	
4	1,2-Diaminobenzene(+ S9)	95-54-5	6.60E-05	8.71E-05	7.06E-04	7.06E-04	-3.15	SRC
5	1,2-Dimethylhydrazine	540-73-8	6.82E-03			6.82E-03	-2.17	
6	1,2-Epoxybutan	106-88-7	2.08E+01	1.70E+01	1.77E+01	1.77E+01	1.25	SRC
7	1,3-Dinitropyrene	75321-20-9	1.27E-05	1.99E-05		1.27E-05	-4.90	
8	1,3-Dioxane	505-22-6	2.90E+00	2.95E+00		2.90E+00	0.46	
9	1,5-Dinitronaphthalene	605-71-0	8.03E-04	2.51E-03		8.03E-04	-3.10	
10	1,6-Dinitropyrene	42397-64-8	1.27E-05	1.99E-05		1.27E-05	-4.90	
11	1,8-Dinitropyrene	42397-65-9	1.27E-05	1.99E-05		1.27E-05	-4.90	
12	1-Bromo pentane	110-53-2	2.59E+03	2.24E+03	1.93E+03	1.93E+03	3.29	SRC
13	1-Chloro-2,4-dinitrobenzene	97-00-7	6.10E-03	3.09E-02		6.10E-03	-2.21	
14	1-Naphthylamine	134-32-7	1.82E-02	1.48E-02	1.09E-02	1.09E-02	-1.96	ABRAHAM,MH ET AL. (1994)
15	1-Nitronaphthalene	86-57-7	2.04E-01	3.02E-01	1.73E-01	1.73E-01	-0.76	ALTSCHUH,J ET AL. (1999)
16	1-Nitropyrere	5522-43-0	3.21E-03	2.40E-03		3.21E-03	-2.49	
17	2,4,7-Trinitro-9-fluorene	34263-36-0	1.00E-06	1.95E-06		1.00E-06	-6.00	
18	2,4-Diaminotoluene (+ S9)	95-80-7	7.29E-05	9.34E-05		7.29E-05	-4.14	
19	2,4-Dimethyl-1,3-dioxane	766-20-1	5.12E+00	1.86E+00		5.12E+00	0.71	
20	2,4-Dinitrotoluene	121-14-2	9.08E-03	3.89E-02	5.30E-03	5.30E-03	-2.28	ALTSCHUH,J ET AL. (1999)
21	2,6-Diaminotoluene (+ S9)	823-40-5	7.29E-05	9.34E-05		7.29E-05	-4.14	
22	2,7-Dinitro-8-fluorenone	31551-45-8	1.03E-06			1.03E-06	-5.99	
23	2-Acetylaminofluorene (+S9)	53-96-3	1.88E-05			1.88E-05	-4.73	

Nr.	Compound	CAS.Reg.No.	H bond	H group	H exp	H selected	LogH selected	Ref HLC
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	77094-11-2	3.82E-08			3.82E-08	-7.42	
25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9)	76180-96-6	1.79E-09			1.79E-09	-8.75	
26	2-Amino-3-methyl-9H-pyrido(2,3-b)indole; MeA alpha C (+ S9)	68006-83-7	4.32E-09			4.32E-09	-8.36	
27	2-Amino-6-methyidipyrido(1,2-a:3',2'd)imidazole (Glu-P-l;+ S9)	67730-11-4	1.11E-10			1.11E-10	-9.96	
28	2-Amino-9H-pyrido(2,3-b)indole (+S9)(A alpha C)	26148-68-5	3.92E-09			3.92E-09	-8.41	
29	2-Aminoanthracene(+ S9)	613-13-8	1.78E-03	1.02E-03		1.78E-03	-2.75	
30	2-Amino-dipyrido(1,2-a:3',2'-d)imidazole-2-amine (Glu-P-2; + S9)	67730-1 0-3	1.00E-10			1.00E-10	-10.00	
31	2-Aminofiuorene(+S9)	153-78-6	4.45E-03	1.38E-03		4.45E-03	-2.35	
32	2-Amino-l-methyl-6-phenylimidazo[4,5-b]pyridine (PhlP)	105650-23-5	2.73E-08			2.73E-08	-7.56	
33	2-Nitrofluorene	607-57-8	6.47E-02	2.81E-02		6.47E-02	-1.19	
34	2-Nitronaphthalene	581-89-5	2.04E-01	3.02E-01		2.04E-01	-0.69	
35	2-Nitro-p-phenylenediamine	5307-14-2	5.70E-06	7.25E-07		5.70E-06	-5.24	
36	3,7-Dinitrofluoranthene	105735-71-5	1.27E-05	1.99E-05		1.27E-05	-4.90	
37	3,9-0initrofluoranthene	22506-53-2	1.27E-05	1.99E-05		1.27E-05	-4.90	
38	3-Amino-1,4-dimethyl.SH-pyrido[3,4-b]lindole; Trp-P-1 (+ S9)	62450-06-0	4.78E-09			4.78E-09	-8.32	
39	3-Amino-l-methyl-5H-pyrido[4,3-b]indole; Trp-P 2 (+ S9)	62450-07-1	4.32E-09			4.32E-09	-8.36	

Nr.	Compound	CAS.Reg.No.	H bond	H group	H exp	H selected	LogH selected	Ref HLC
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	77094-11-2	3.82E-08			3.82E-08	-7.42	
25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9)	76180-96-6	1.79E-09			1.79E-09	-8.75	
40	3-Methoxy-4-aminoazobenzene(+S9)	3544-23-8	3.01E-05	5.01E-04		3.01E-05	-4.52	
41	3-Methylcholanthrene (+ S9)	56-49-5	2.91E-01	2.95E-02		2.91E-01	-0.54	
42	3-Nitrofluoranthene	892-21-7	3.21E-03	2.40E-03		3.21E-03	-2.49	
43	4,4'-Dinitrobiphenyl	1528-74-1	6.32E-04	2.88E-03		6.32E-04	-3.20	
44	4-Aminobiphenyl	92-67-1	1.43E-02	1.70E-02		1.43E-02	-1.84	
45	4-Methyl-1,3-dioxane	1120-97-4	3.85E+00	6.91E+00		3.85E+00	0.59	
46	4-Nitro-o-phenylenediamine	99-56-9	2.60E-07	7.25E-07		2.60E-07	-6.59	
47	4-Nitroquinoline-N-oxide	56-57-5	2.67E-09			2.67E-09	-8.57	
48	4-Nitrosodiphenylamine	156-10-5	1.08E-03			1.08E-03	-2.97	
49	4-Nitroso-N,N-dimethylaniline	138-89-6	8.79E-02			8.79E-02	-1.06	
50	5-Butyrolactone	36536-46-6	1.01E+01	2.95E+00		1.01E+01	1.00	
51	5-Fluorouracil	51-21-8	1.63E-05			1.63E-05	-4.79	
52	5-MOP (+ UV)	484-20-8	3.92E-03			3.92E-03	-2.41	
53	5-Nitro-2-furaldehyde	698-63-5	5.19E-03			5.19E-03	-2.29	
54	5-Nitro-2-furylacrylacid	710-25-8	1.02E-07			1.02E-07	-6.99	
55	5-Nitroacenaphthene	602-87-9	1.09E-01	4.79E-02		1.09E-01	-0.96	
56	6-Aminochrysene(+ S9)	2642-98-0	1.74E-04	7.08E-05		1.74E-04	-3.76	
57	6-chloro-9-(3-(2-chloroethylamino)propyl)amino-2-rnethoxyacridine*2HCl(ICR-191)	17070-45-0	4.17E-11			4.17E-11	-10.38	
58	6-Nitrochrysene	2-89-6	1.90E-14			1.90E-14	-13.72	
59	7,12-Dimethylbanzanthracene (+ S9)	57-97-6	5.98E-01	1.99E-01		5.98E-01	-0.22	
60	8-MOP (+ UV)	298-81-7	3.92E-03			3.92E-03	-2.41	

Nr.	Compound	CAS.Reg.No.	H bond	H group	H exp	H selected	LogH selected	Ref HLC
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	77094-11-2	3.82E-08			3.82E-08	-7.42	
25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9)	76180-96-6	1.79E-09			1.79E-09	-8.75	
61	8-Proplotactone	57-57-8	7.58E+00	1.26E+00		7.58E+00	0.88	
62	9 Aminoacridine	90-45-9	2.32E-06	1.59E-05		2.32E-06	-5.63	
63	Acridine Orange	494-38-2	1.67E-06			1.67E-06	-5.78	
64	Acrinol(+S9)	1837-57-6	6.46E-11	7.08E-09		6.46E-11	-10.19	
65	Adriamycin(Adr)	25316-40-9	2.19E-18			2.19E-18	-17.66	
66	Afiatoxin G1	1165-39-5	4.86E-08			4.86E-08	-7.31	
67	Aflatoxin B1	1162-65-8	1.37E-08			1.37E-08	-7.86	
68	Amphotericin B	1397-89-3	2.07E-30			2.07E-30	-29.68	
69	Amsacrine	51264-14-3	1.65E-11			1.65E-11	-10.78	
70	Auramine	2465-27-2	3.57E-04			3.57E-04	-3.45	
71	Avarol(+ S9)	55303-98-5	6.73E-05			6.73E-05	-4.17	
72	Azaserine	115-02-6	3.19E-06			3.19E-06	-5.50	
73	Azinphos-Methyl	86-50-0	2.80E-05		2.34E-03	2.34E-03	-2.63	SRC
74	Azobenzene(+ S9)	103-33-3	1.44E+00	1.51E+00	1.32E+00	1.32E+00	0.12	SRC
75	Benz(a)anthracene(+ S9)	56-55-3	4.91E-01	1.74E-01	1.18E+00	1.18E+00	0.07	BAMFORD,HA ET AL. (1999)
76	Benzidine (+ S9)	92-87-5	5.07E-06	6.91E-06		5.07E-06	-5.29	
77	Benzo(a)pyrene(+ S9)	50-32-8	7.94E-02	1.99E-02	4.48E-02	4.48E-02	-1.35	TEN HULSCHER,TEM ET AL. (1992)
78	Benzylchloride	100-44-7	2.05E+02	3.89E+01	4.04E+01	4.04E+01	1.61	SRC
79	Bleomycin	11056-06-7						
80	Bromo acetic acid	79-08-3	6.19E-03	8.71E-03		6.19E-03	-2.21	
81	Bromobenzene	108-86-1	2.11E+02	2.19E+02	2.42E+02	2.42E+02	2.38	SHIU,WY & MACKAY,D (1997)
82	Captan	133-06-2	4.50E-04		6.86E-04	6.86E-04	-3.16	SRC

Nr.	Compound	CAS.Reg.No.	H bond	H group	H exp	H selected	LogH selected	Ref HLC
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	77094-11-2	3.82E-08			3.82E-08	-7.42	
25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9)	76180-96-6	1.79E-09			1.79E-09	-8.75	
83	Chloramine T	127-65-1	8.45E-01			8.45E-01	-0.07	
84	Chromium(VI)oxide	1333-82-0	0.00E+00	0.00E+00				
85	Chrysene (+ S9)	218-01-9	4.91E-01	1.74E-01	5.13E-01	5.13E-01	-0.29	BAMFORD,HA ET AL. (1999)
86	Ciprofloxacin	85721-33-1	4.99E-14			4.99E-14	-13.30	
87	cis-Platinum	15663-27-1	#VALUE!			0.00E+00		
88	Citrinin	518-75-2	2.06E-10			2.06E-10	-9.69	
89	Cumenehydroperoxide	80-15-9	9.51E-02		2.12E-02	2.12E-02	-1.67	SRC
90	Cupferron	135-20-6	3.55E-04			3.55E-04	-3.45	
91	Danthron	117-10-2	5.34E-06			5.34E-06	-5.27	
92	Daunomycin	23541-50-6	1.40E-20			1.40E-20	-19.85	
93	Daunorubicin	20830-81-3	1.40E-20			1.40E-20	-19.85	
94	Dibromoacetonitrile	3252-43-5	3.98E-02			3.98E-02	-1.40	
95	Dichlofluanid	1085-98-9	6.61E-02		3.71E-03	3.71E-03	-2.43	SRC
96	Diethylnitrosamine (+ S9)	55-18-5	3.56E-01	1.70E-01	3.56E-01	3.56E-01	-0.45	MIRVISH,SS ET AL. (1976)
97	Diethylsulfate (+ S9)	64-67-5	4.50E-01		8.24E-01	8.24E-01	-0.08	SRC
98	Dimethylnitrosamine (+ S9)	62-75-9	2.02E-01	1.18E-01	1.78E-01	1.78E-01	-0.75	MIRVISH,SS ET AL. (1976)
99	Dimethylsulfate	77-78-1	2.55E-01		3.92E-01	3.92E-01	-0.41	SRC
100	Dimethylsulfoxide	67-68-5	4.86E-03	1.86E+02	1.48E-04	1.48E-04	-3.83	TAFT,RW ET AL. (1985)
101	Dithianone	3347-22-6	3.81E-12		5.54E-06	5.54E-06	-5.26	SRC
102	Doxorubicinhydrochloride	25316-40-9	0.00E+00	0.00E+00				
103	Ellipticine	519-23-3	1.32E-06	4.46E-06		1.32E-06	-5.88	
104	Enoxacin	74011-58-8	1.12E-16			1.12E-16	-15.95	
105	Epichlorhydrin	106-89-8	5.51E+00	2.57E-01	2.98E+00	2.98E+00	0.47	SRC
106	Epoxystyrene	96-09-3	9.51E-01		1.55E+00	1.55E+00	0.19	SRC

Nr.	Compound	CAS.Reg.No.	H bond	H group	H exp	H selected	LogH selected	Ref HLC
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	77094-11-2	3.82E-08			3.82E-08	-7.42	
25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9)	76180-96-6	1.79E-09			1.79E-09	-8.75	
107	Ethidium bromide (+ S9)	1239-45-8	1.78E-15			1.78E-15	-14.75	
108	Ethyl methanesulfonate (EMS)	62-50-0	5.25E-01		2.54E-02	2.54E-02	-1.60	SRC
109	Ethylene dibromide	106-93-4	1.27E+02	1.51E+01	6.54E+01	6.54E+01	1.82	SRC
110	Folpet	133-07-3	1.51E-04		7.51E-03	7.51E-03	-2.12	SRC
111	Formaldehyde	50-00-0	9.11E+00	6.02E+00	3.30E-02	3.30E-02	-1.48	BETTERTON,EA & HOFFMAN,MR (1988)
112	Furazolidone	67-45-8	3.20E-06			3.20E-06	-5.50	
113	Furylfuramide (AF-2)	3688-53-7	4.56E-09			4.56E-09	-8.34	
114	Glutaraldehyde	111 30-8	1.08E-02	2.34E-03		1.08E-02	-1.97	
115	Glyoxal	4405-13-4	3.14E-13	9.12E-19		3.14E-13	-12.50	
116	Harmane	486-84-0	1.23E-05	7.77E-05		1.23E-05	-4.91	
117	Hydrazine sulfate (+ S9)	10034-93-2	8.55E-09			8.55E-09	-8.07	
118	Hydrogen peroxide	7722-84-1	2.09E-03			2.09E-03	-2.68	
119	Hydroxy urea	127-07-1	5.32E-06			5.32E-06	-5.27	
120	m-Dinitrobenzene	99-65-0	8.23E-03	3.63E-02	4.81E-03	4.81E-03	-2.32	ALTSCHUH,J ET AL. (1999)
121	Methapyrilene (+ S9)	91-80-5	3.18E-07			3.18E-07	-6.50	
122	Methyl methanesulfonate (MMS)	66-27-3	3.95E-01			3.95E-01	-0.40	
123	Methylbromide	74-83-9	8.33E+02	6.17E+02	6.12E+02	6.12E+02	2.79	SRC
124	Methylene bromide	74-95-3	9.59E+01	8.51E+01	8.06E+01	8.06E+01	1.91	MOORE,RM ET AL. (1995)
125	Methylenebromide	74-95-3	9.59E+01	8.51E+01	8.06E+01	8.06E+01	1.91	MOORE,RM ET AL. (1995)
126	Metronidazol	443-48-1	1.66E-06			1.66E-06	-5.78	
127	Mitomycin C	50-07-7	1.12E-19			1.12E-19	-18.95	
128	Nalidixic acid (free acid)	389-08-2	5.02E-11			5.02E-11	-10.30	
129	Nalidixic acid (sodium-salt)	3374-05-8	5.04E-11			5.04E-11	-10.30	
130	N-Ethyl-N*-nitro-N-	4245-77-6	1.59E-07			1.59E-07	-6.80	

Nr.	Compound	CAS.Reg.No.	H bond	H group	H exp	H selected	LogH selected	Ref HLC
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	77094-11-2	3.82E-08			3.82E-08	-7.42	
25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9) nitrosoguanidine	76180-96-6	1.79E-09			1.79E-09	-8.75	
131	N-Ethyl-N-nitrosourea	759-73-9	1.29E-05			1.29E-05	-4.89	
132	Nifuroxazide	965-52-6	1.92E-11			1.92E-11	-10.72	
133	Nitrofurantoin	67-20-9	1.30E-07			1.30E-07	-6.88	
134	Nitrofurazone	59-87-0	3.04E-08			3.04E-08	-7.52	
135	Nitrogen dioxide	10102-44-0						
136	N-Methyl-N-nitro-N-nitrosoguanidine	70-25-7	1.20E-07			1.20E-07	-6.92	
137	N-Methyl-N-nitrosourea	684-93-5	9.72E-06			9.72E-06	-5.01	
138	N-Nitrosobutylurea	869-01-2	2.28E-05			2.28E-05	-4.64	
139	N-Nitrosocimetidine	73785-40-7	0.00E+00	0.00E+00				
140	N-Nitrosodiethanolamine	1116-54-7	4.76E-07	2.24E-11		4.76E-07	-6.32	
141	N-Nitrosodiphenyiamine	86-30-6	1.19E-01			1.19E-01	-0.93	
142	N-Nitroso-N-butyl-N-propylamine	25413-64-3	8.32E-01	4.79E-01		8.32E-01	-0.08	
143	Norharmane	244-63-3	1.11E-05	5.62E-05		1.11E-05	-4.96	
144	Nystatin	1400-61-9	1.62E+00	1.99E-02		1.62E+00	0.21	
145	o-Aminoazotoluene	97-56-3	6.21E-04	7.08E-04	3.12E-03	3.12E-03	-2.51	SRC
146	Ofloxacin	82419-36-1	4.88E-15		0.00E+00	4.88E-15	-14.31	
147	o-Nitroanisol	91-23-6	1.24E-01	3.55E+00	4.21E-02	4.21E-02	-1.38	SRC
148	o-Tolidine	119-93-7	6.17E-06	7.94E-06		6.17E-06	-5.21	
149	Paraquat	4685-14-7	3.16E-08			3.16E-08	-7.50	
150	Phenobarbital	50-06-6	1.62E-09			1.62E-09	-8.79	
151	Phenylhydrazine	100-63-0	7.80E-04		4.33E-04	4.33E-04	-3.36	SRC
152	Pikrinic acid (+ S9)	88-89-1	1.07E-05	3.72E-08	1.67E-06	1.67E-06	-5.78	SRC
153	Potassium chromate	11073-34-0						

Nr.	Compound	CAS.Reg.No.	H bond	H group	H exp	H selected	LogH selected	Ref HLC
24	2-Amino-3,4-dimethyl-3H-imidazol(4,5-f)quinoline; MelQ (+ S9)	77094-11-2	3.82E-08			3.82E-08	-7.42	
25	2-Amino-3-methyl-3H-imidazo(4,5-f)quinoline; IQ (+ S9)	76180-96-6	1.79E-09			1.79E-09	-8.75	
154	Potassium dichromate (Cr207 2-)	7778-00-9						
155	p-Phenylendiamine	106-50-3	6.60E-05	8.71E-05		6.60E-05	-4.18	
156	Propane sultone	1120-71-4	2.31E-01			2.31E-01	-0.64	
157	Propylene oxide	75-56-9	1.57E+01	1.21E+01	6.83E+00	6.83E+00	0.83	SRC
158	Pyrogallol	87-66-1	5.95E-10	9.77E-10		5.95E-10	-9.23	
159	Quercetin(+ S9)	117-39-5	6.47E-16			6.47E-16	-15.19	
160	Saccharin	81-07-2	1.21E-04			1.21E-04	-3.92	
161	Selene dioxide	7446-08-4						
162	Sodium nitrite(NO2-)	7632-00-0						
163	Sterigmatocystin	10048-13-2	2.78E-07			2.78E-07	-6.56	
164	Streptonigrin	3930-19-6	7.08E-25			7.08E-25	-24.15	
165	Streptozotocin	18883-66-4	7.70E-17			7.70E-17	-16.11	
166	Styrene oxide	96-09-3	9.51E-01		1.55E+00	1.55E+00	0.19	SRC
167	t-Butylhydroperoxide	75-91-2	1.57E+00			1.57E+00	0.20	
168	Trichloroacetone	918-00-3	2.13E-01			2.13E-01	-0.67	
169	Trichloronitromethane	76-06-2	1.80E-01		2.01E+02	2.01E+02	2.30	KAWAMOTO,K & URANO,K (1989)
170	Tris(2,3-dibromopropyl)phosphate	126-72-7	3.14E-07			3.14E-07	-6.50	

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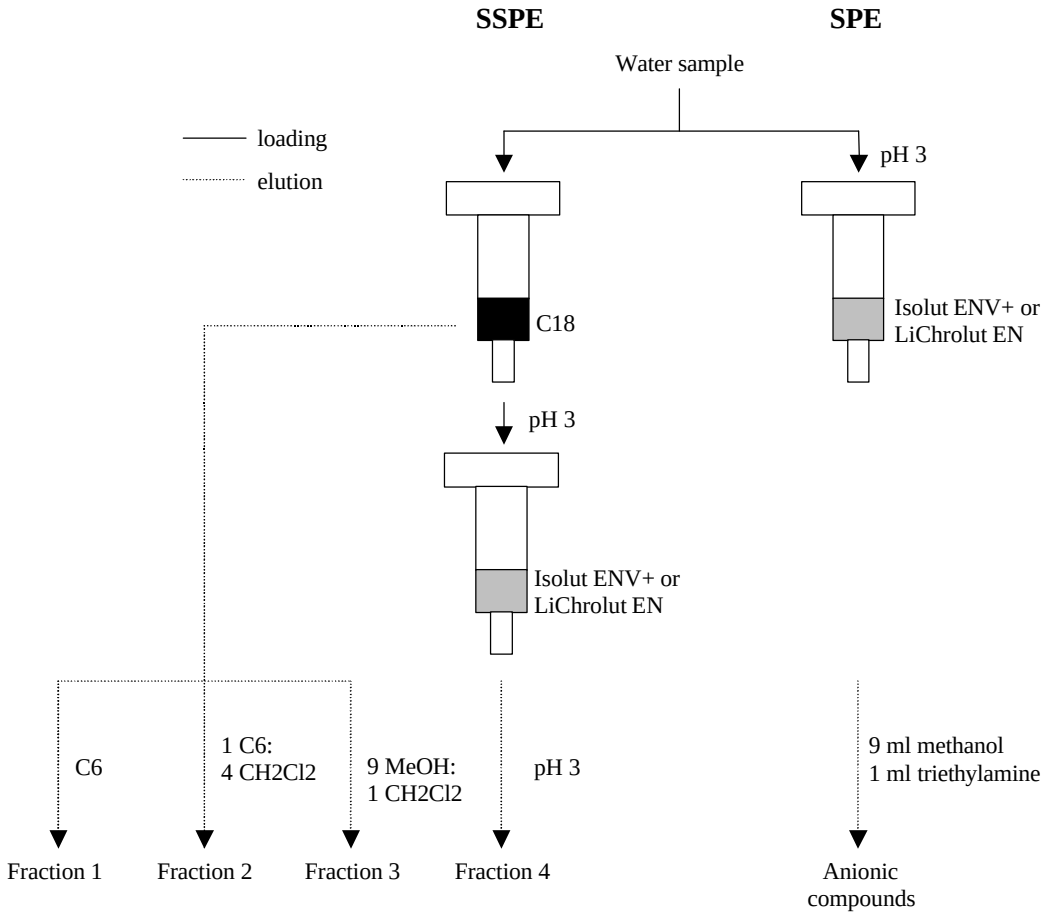
Annex 5 Detailed description of the selected SPE approaches

C18 + Isolut ENV+	**Description**
Water samples	500 ml or more: filtration if needed with 0.45 mm membrane filters and pH adjustment (pH 7.0). Add some 10% of methanol to the sample to improve the desorption of hydrophobic compounds.
C18 silica cartridge	500 mg sorbent in 6 ml cartridge.
Conditioning	7 ml methanol and then 3 ml of HPLC-water at 1 ml/min: the sorbent is not allowed to become dry before the percolation of the sample.
Percolation	Percolation of the filtered sample (pH 7.0) into the C18 cartridge at 5 ml/min.
Drying	After sample percolation, the sorbent is completely dried under vacuum for 20–30 min.
Desorption	Differential elution can be applied to C18 cartridge to obtain three fractions (F) containing contaminates with different polarities and functional groups: F1: 2x5 ml hexane; F2: 2x5 ml dichloromethane:hexane (4:1, v/v) and F3: methanol:dichloromethane (9:1, v/v). If this fractionation is not needed, the desorption can be done only with F3. Wait 1–5 min between the two aliquots (2x5 ml) to allow sufficient contact time between the solvent and the trapped analytes.
Isolut ENV+ or LiChrolut	Same conditioning as by C18 cartridge. The residual (the C18-preconcentrated) water is acidified to pH 3–3.5 and loaded onto the Isolut ENV+ cartridge (200 mg, 6 ml) at 15 ml/min
Drying	After sample percolation, the sorbent is completely dried under vacuum for 20–30 min.
Desorption	Desorption by 2x5 ml of methanol or 2x5 ml of 1 ml (5 mM triethylamine and 5 mM acetic acid, pH=6.5) and 9 ml of methanol at 1 ml/min waiting 5 min between the two aliquots to allow sufficient contact time between the solvent and the trapped analytes.
Evaporation	Combine all the extracts and evaporate under a gentle stream of nitrogen to the desired volume (0.5 ml). Before starting the evaporation, add some dimethyl sulfoxide (DMSO) to avoid loss of volatiles during evaporation.
UmuC-test	The extract is ready for the UmuC-test.

C18 + GCB	**Description**
Water samples	500 ml or more: filtration if needed with 0.45 mm membrane filters and pH adjustment (pH 7.0). Add some 10% of methanol to the sample to improve the desorption of hydrophobic compounds.
C18 silica cartridge	500 mg sorbent in 6 ml cartridge.
Conditioning	10 ml methanol and then 10 ml of HPLC-water at 1 ml/min: the sorbent is not allowed to become dry before the percolation of the sample.
Percolation	Percolation of the filtered sample (pH 7.0) into the C18 cartridge at 5 ml/min.
Drying	After sample percolation, the sorbent is completely dried under vacuum for 20–30 min.
Desorption	Differential elution can be applied to C18 cartridge to obtain three fractions (F) containing contaminates with different polarities and functional groups: F1: 2x5 ml hexane; F2: 2x5 ml dichloromethane:hexane (4:1, v/v) and F3: methanol:dichloromethane (9:1, v/v). If this fractionation is not needed, the desorption can be done only with F3. Wait 1–5 min between the two aliquots (2x5 ml) to allow sufficient contact time between the solvent and the trapped analytes.
GCB (carbograph 4)	Same conditioning as by C18 cartridge. The only difference is that HPLC-water was acidified to pH 2-3. The residual (the C18-preconcentrated) water is acidified to pH 3–3.5 and loaded onto the carbon cartridge (500 mg, 6 ml) at 10 ml/min
Drying	After sample percolation, the sorbent is completely dried under vacuum for 20–30 min.
Desorption	Desorption by 2x5 ml of dichloromethane:methanol (80:20, v/v) mixture at 1 ml/min waiting 5 min between the two aliquots to allow sufficient contact time between the solvent and the trapped analytes.
Evaporation	Combine all the extracts and evaporate under a gentle stream of nitrogen to the desired volume (0.5 ml). Before starting the evaporation, add some dimethyl sulfoxide (DMSO) to avoid loss of volatiles during evaporation.
UmuC-test	The extract is ready for the UmuC-test.



Examples of SPE and SSPE approaches.