# Sampling of pesticides in water using solid-phase extraction

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by

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## Abstract

The aim of this study was to develop a sampling method for pesticide residues in water using solid-phase extraction. A sampling equipment has been assembled constituting of a raft, a filter holder with glass micro fibre filter, and an ENV+ solid-phase column connected to a vacuum flask and a pump with reversed function. The equipment can easily be brought out in the field and sampling of a volume of 500-1000 ml can be carried out within 30 minutes. Water soluble and particle bound residues are separated. By using the solid-phase sampling equipment losses of analytes through degradation and adsorption can be reduced and transport of samples to the laboratory is facilitated. The dissolved fraction of a pesticide is captured in the solid-phase column and a pre-washing step of ENV+ is generally needed before use but activation of the columns can be excluded. Elution with dichloromethane can be made directly without a soaking step. The particle bound fraction collected on the glass micro fibre filter is efficiently extracted when using Soxtec extraction with dichloromethane/acetone (1:1, v/v).
In the beginning of the study focus was on two pyrethroids, esfenvalerate and deltamethrin, since these hydrophobic substances can be difficult to recover from the solid-phase column. When appropriate extraction conditions were achieved for the two pyrethroids 14 other pesticides of different character were introduced in the study to investigate whether the selected extraction methods gave satisfactory recoveries. Adequate results (total recovery between 70 and 130%) were achieved for the following pesticides: atrazine, deltamethrin, diflufenican, diuron, esfenvalerate, ethofumesate, hexazinon, isoproturon, lambda-cyhalotrin, metazachlor, pirimicarb, propiconazole and simazine. The recovery of terbutylazine exceeded 130% while the recovery of fenpropimorph and metamitron were lower than 70%. Esfenvalerate, deltamethrin and lambda-cyhalothrin were to a large extent recovered from particles while the major portion of other pesticides studied were retrieved from the water phase.

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# Sampling of pesticides in water using solid-phase extraction

## 1. Aim

The aim of this study was to develop a sampling method where solid-phase extraction can be used in the field to take water samples for pesticide analysis.

## 2. Introduction

Pesticides may appear in surface waters as a result of e.g. surface run-off from agricultural fields. The amount of pesticides transported to surface waters depends on factors such as soil characteristics, topography, weather, agricultural practices and the chemical properties of the pesticides (Albanis *et al.* 1998). Many pesticides are highly toxic and may cause serious damages to aquatic ecosystems. It is therefore of great importance to have methods for quantification of these substances and thereby be able to evaluate the possible risk for ecotoxicological damages.

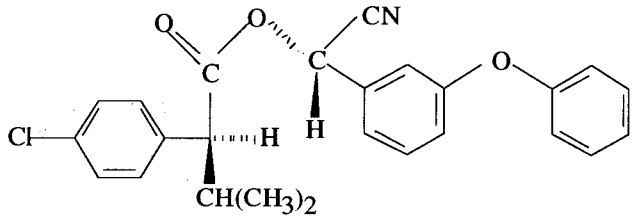
Water samples for pesticide analysis are usually collected in glass bottles which then are sent to the laboratory. These bottles are quite troublesome to transport due to their size, weight and fragility. Some pesticides may be adsorbed to the glass of the sample bottles or be degraded on the way to the laboratory, which leads to losses of the analytes (Woin, 1994). A sampling method for pesticides in water where solid-phase extraction can be used directly in field is a way of overcoming these problems.

Solid-phase extraction (SPE) is a widely used technique for the isolation of pesticides. The technique is based on the principles of liquid chromatography. The analytes are distributed between a solid stationary and a liquid mobile phase and are thereby retained. SPE has become an important alternative to liquid-liquid extraction (LLE) due to its several advantages. SPE is easier to perform, less time consuming, more selective and the consumption of organic solvents is drastically reduced compared to LLE. SPE has many applications: sample preparation, purification, trace enrichment and class fractionation. An earlier study of the possibility to use SPE for sampling been performed by P. Woin (1994).

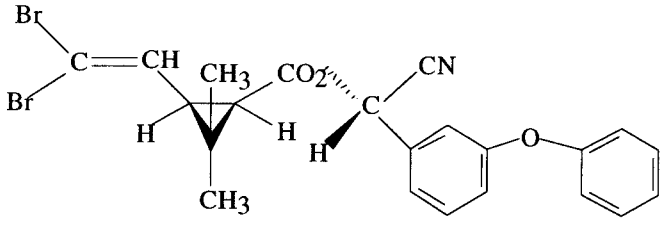
There are many different types of SPE columns. In this study a highly cross-linked polystyrene divinylbenzene polymer ENV+ was used as sorbent. It was selected since it has the ability to retain substances over a wide range of polarity. ENV+ consists of a polymeric material and these kinds of sorbents have a higher specific surface area compared to the more commonly used silica based sorbents, which gives a larger adsorption efficiency. Also the flow characteristics of this polymeric sorbents is better allowing a higher flow rate which makes them more suitable for environmental samples (Junker-Buchheit, 1996). The silica base has also the disadvantage that it may hydrolyse in alkaline waters (pH $\geq$ 8), whereby the carbon chains are lost which leads to reduced extraction efficiency in that kind of environment (Henrik Kylin, personal communication).

The present study is reported in four different sections. The first two sections account for the experimental work limited to two test substances: deltamethrin and esfenvalerate. Here the SPE conditions and the extraction of the particle bound fraction were studied more closely. The third section involved a number of pesticides to examine whether the developed methods were more generally applicable to substances of different character. In the last part of the study a sampling equipment and its performance in a field study is presented.

The pyrethroids, esfenvalerate and deltamethrin, were selected for the initial study (figure 1a and b). Pyrethroids is a group of pesticides that are among the most toxic for aquatic organisms such as fish and crustaceans (van der Hoff *et al.* 1996). It is therefore of great interest to quantify the presence of these substances. Pyrethroids are highly lipophilic and tend to adsorb to the glass of the sampling bottles. This makes solid-phase extraction directly in the field specially important to avoid sample losses due to adsorption. Pyrethroids, however, have been regarded as "difficult" in SPE applications. They tend to sorb so strongly that it is hard to elute them (Pihlström *et al.* 1996). Different SPE conditions were therefore studied within the aim to achieve an acceptable recovery.




a)




b)

Figure 1. Structural formula of a) esfenvalerate and b) deltamethrin

The distribution of a pesticide between particulate matter and the water phase is important to consider from an analytical and an ecotoxicological point of view. If water samples are filtered without extraction of particulate matter considerable amounts of a substance can be lost. LLE of unfiltered water samples may give low recoveries of particle bound residues. The same problem may appear when using SPE and in addition the sorbent is easily clogged by particles. Aquatic organisms are not only exposed to the water soluble fraction of a pesticide but also to the particle bound fraction, through food intake, respiration and direct contact (Woin, 1994). Hydrophobic substances such as esfenvalerate and

deltamethrin have a large affinity for particulate matter. The separation and determination of the particle bound fraction were therefore studied.

The following pesticides were included when the study was expanded to a larger number of test substances: atrazine, deltamethrin, diflufenican, diuron, esfenvalerate, ethofumesate, fenopropimorph, hexazinon, isoproturon, lambda-cyhalotrin, metamitron, metazachlor, pirimicarb, propiconazole, simazine and terbuthylazine. Fenpropimorph and propiconazole are fungicides and deltamethrin, esfenvalerate, lambda-cyhalothrin and pirimicarb are insecticides while the remaining substances are herbicides (Tomlin, 1997). They were selected since they represent a broad range of pesticides of different character. Most of them are frequently used in Sweden and they are relatively easy to determine (Per Woin, personal communication).

Finally a SPE sampling device was developed, and evaluated in the beginning of May 1999 in connection with a field study in Skåne (the most southern part of Sweden). Dr Per Woin and the PhD student Lina Wendt-Rasch at the Department of Chemical Ecology and Ecotoxicology at Lund University are working with ponds in areas of intensive agriculture. They are studying the effects of exposure of pesticides to aquatic ecosystems. The SPE sampling method will be used in these ponds and the results from subsequent analysis will be included in their research.

# 3. Solid-phase extraction studies

## 3.1. Experiment 1a
## Study of the need for activation of ENV+ columns and the distribution of analytes between sampling flasks, SPE columns and flow-through water

The aim of this experiment was to investigate whether activation of the columns influences the recovery of esfenvalerate and deltamethrin. Closer examination of three analytical steps was performed to study where losses of the analytes might appear. The steps studied were (i) the transfer of analytes from the sample bottle to the column, (ii) the solid-phase extraction and (iii) analyte break through.

### *3.1.1. Standards and solvents*

Samples were prepared in tap water by dilution of a stock solution in acetone with a concentration of 1.007 µg/ml of deltamethrin and 0.835 µg/ml of esfenvalerate. The additions of esfenvalerate and deltamethrin to tap water samples in 1 litres glass bottles were made approximately 0.5 h prior to extraction. Each sample had a volume of 1 litre and the final concentration of esfenvalerate and deltamethrin was 0.835 µg/l and 1.007 µg/l, respectively. Ethion was used as internal standard and 0.166 µg from a stock solution in acetone of 4.144 µg/ml was added to the sample prior to extraction. The internal standard is intended to compensate for losses during sample handling and in the final gas chromatographic (GC)-determination. Ethion was chosen since it is not used in Sweden and the chance of finding it in real environmental samples is thereby low. Its peak appears in the middle of the chromatogram and ethion is also detectable by both of the two detectors used for the quantification of pesticides: electron capture detector (ECD) and nitrogen phosphorous detector (NPD). External standards with two different concentrations of esfenvalerate and deltamethrin were used for quantification. All pesticides were provided by Dr. Ehrenstorfer Gmbh, Augsburg, Germany. For the extraction processes dichloromethane, cyclohexane, ethyl acetate and acetone were used. Solvents were provided by KEBO lab, Spånga, Sweden and they were all of pesticide grade.

### *3.1.2. Extraction equipment*

Isolute SPE columns with an internal volume of 6 ml and a content of 200 mg of ENV+ (from Sorbent AB, Västra Frölunda, Sweden) were used. A vacuum manifold (Vac Elute from Analytichem International, nowadays Sorbent AB, Västra Frölunda, Sweden) was used for flow control when needed (figure 2).

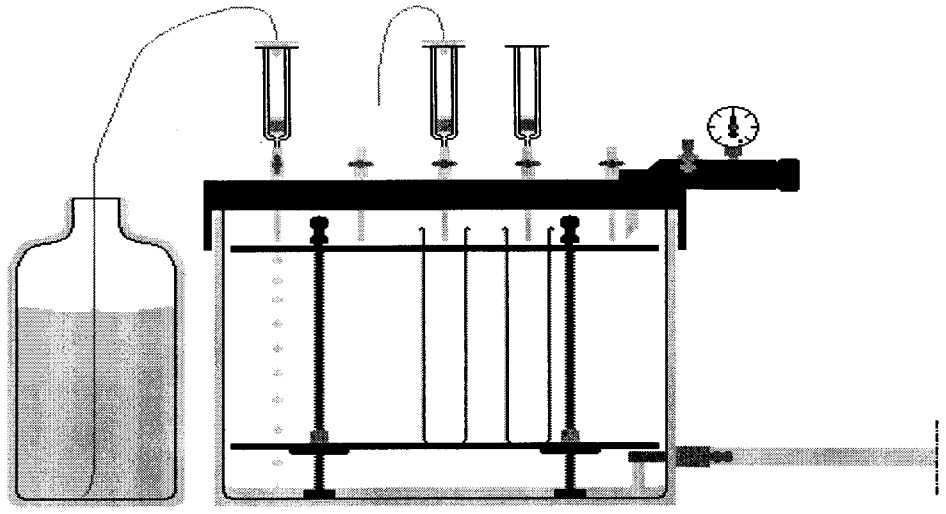


Figure 2. Vacuum manifold. Solid-phase columns were placed on top of the vacuum manifold and teflon tubes were used to connect the water samples to the columns. Suction was achieved by connecting the vacuum manifold to a vacuum source.

### *3.1.3. Activation and application of sample*

Activation of the SPE columns is performed to increase the contact area between the sorbent and the sample (Tomas Popoff, Sorbent AB, personal communication). It is carried out by treating the sorbent with a "matrix-like" solvent and for water samples methanol is usually used. Three columns were activated using 5 ml of methanol that was allowed to flow through the column under self pressure. Ultra pure water (10 ml) was then passed through the column. Three non-activated columns served as control. Spiked tap water samples (1 litre) were led through the columns at a flow rate of approximately 50 ml/min. Flow-through water was collected in a vacuum flask. The sample bottle was then rinsed with 10 ml of ultra pure water that also was passed through the column. To account for possible losses of the analytes the following three analytical steps were studied more closely:

1) The transfer of analytes from sample bottle to columns by washing the emptied bottles with dichloromethane and thereby estimating the amount of analytes that remained adsorbed to the glass of the sample bottle.
2) The SPE columns to see how much of the analytes that were retained and then released from the column.
3) Analyte break through by liquid-liquid extraction of the water collected in the vacuum flask.

### *3.1.4. Bottle wash*

The empty sample bottle was rinsed twice with 50 ml of dichloromethane. Water was removed from the combined dichloromethane volumes by filtration through a filter with sodium sulphate. Cyclohexane (5 ml) was added as a keeper to prevent losses of the analytes during evaporation. The sample was concentrated using a rotary vacuum evaporator until less then 0.5 ml of the extract remained. The volume was adjusted to 0.5 ml with cyclohexane/acetone (9:1).

*3.1.5. Solid-phase extraction*

The columns were put under vacuum until dryness. Analytes were eluted from the columns using 2x3 ml of ethyl acetate/acetone (1:1, v/v). The extract was dried through a filter with sodium sulphate. Cyclohexane (5 ml) was added and the sample was concentrated using a rotary vacuum evaporator until less then 2 ml of the extract remained. The volume was adjusted to 2 ml with cyclohexane/acetone (9:1).

*3.1.6. Liquid-liquid extraction of the water that has passed the columns*

The water sample that had passed the column was shaken in a separation funnel with three portions of dichloromethane (50 ml). To enhance the extraction saturated sodium chloride solution (50 ml) was added to the water. The organic phase was then dried with sodium sulphate. Cyclohexane (5 ml) was added and the sample was concentrated using a rotary vacuum evaporator until less 2 ml of the extract remained. The volume was adjusted to 2 ml with cyclohexane/acetone (9:1).

*3.1.7. Instrumental and chromatographic conditions*

Samples were analysed on a Hewlett Packard model 5890 gas chromatograph equipped with two $^{63}$Ni electron-capture detectors and two columns (CP-Sil 19 CB and CP-Sil 5 CB with dimensions of 20 m x 0.32 mm i.d. and 0.25 µm film thickness provided by Chrompack Sverige AB, Nacka, Sweden) attached to the same injector. The injection volume was 2 µl and the injection was splitless. Injector temperature was 250 °C and detector temperature was 300 °C. Oven temperature was set to 90 °C for 1 min, increasing 30 °C/min to 180 °C and then 4 °C/min to 260 °C, where it was held for 12 min. Standards were injected every sixth sample. Results were evaluated by calculating a mean value of the responses of the standard in the beginning and in the end of every six samples series. The one of the two standards that was nearest in magnitude to each of the samples was used for quantification. The results were reported as the mean values of the response from the two GC-columns (CP-Sil 19 CB and CP-Sil 5 CB). Esfenvalerate and deltamethrin have isomers that give rise to two peaks of each substance. The responses of the two peaks were added before calculations were carried out. The results were expressed as percentage of the initial amount of deltamethrin and esfenvalerate that were found in each fraction of the sample (recovery). Recoveries of deltamethrin and esfenvalerate from solid-phase extraction were corrected for the recovery of internal standard ethion.

### 3.2. Experiment 1b
### Study of different eluents and elution techniques and the effect of pre-washing of columns

The aim of this experiment was to study the recovery of deltamethrin and esfenvalerate using dichloromethane and ethyl acetate as eluents, in comparison to ethyl acetate/acetone (1:1, v/v). A pre-washing step where ENV+ was treated with dichloromethane and acetone was also tested and evaluated. The pre-washing might be necessary to remove substances from the column material that can disturb the final analysis.

#### *3.2.1. Standards and solvents*

The same type of samples, solvents and internal standard were used as in experiment 1a. Three new standard solutions of different concentrations of deltamethrin and esfenvalerate were prepared in cyclohexane/acetone (9:1, v/v) from the same stock solution as was used to prepare the samples.

#### *3.2.2. Extraction equipment*

The same type of columns and vacuum manifold were used as in experiment 1a.

#### *3.2.3. Solid-phase extraction*

For each eluent six spiked water samples and a blank sample (tap water and internal standard) were led through non-activated columns. Analytes were eluted from the columns using ethyl acetate or dichloromethane (2x3 ml) as eluents. In six of the columns (three for each solvent) analytes were eluted directly while in the six others eluents were allowed to soak into the sorbents for 10 minutes before elution. The rest of the solid-phase extraction procedure was performed as described above.

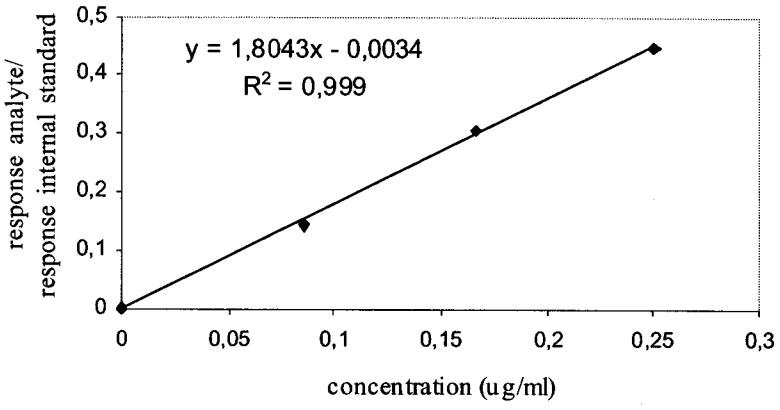
#### *3.2.4. Pre-washing*

The pre-washing was carried out by treating ENV+ columns with two portions of dichloromethane (5 ml) followed by acetone (5 ml). Ultra pure water (10 ml) were then led through the columns. Two columns were pre-washed and two untreated columns were used as control. Dichloromethane was used as eluent and the rest of the sample preparation was following the previous procedure.

#### *3.2.5. Instrumental and chromatographic conditions*

The same instrument and chromatographic conditions were used for analysis as in experiment 1a. Results were evaluated from calibration curves where analyte concentration was a function of response of standards divided by the response of the internal standard (figure 3).

Figure 3. Calibration curve for evaluation of analyte concentration.




## 3.3. Results and discussion

### *3.3.1. Experiment 1a*

Approximately 4% of the added amount of esfenvalerate and deltamethrin seemed to be left on the walls of the bottle (table 1). The large variation of these results may be caused by differences in structure on the glass surface. A scratched surface might increase the adsorption of the analytes. Generally, pyrethroids are known to adsorb strongly to glass and lower adsorption can be due to that the process is time-dependent (Woin, 1994). The short equilibration time (0.5 h) can be an explanation for the low recoveries. The results from liquid-liquid extractions of the flow-through fraction showed that there are approximately 9% break through of esfenvalerate and deltamethrin. Summation of recoveries of deltamethrin and esfenvalerate in the three fractions showed that approximately 50% of the analytes are left on column. Thus the eluent, ethyl acetate/acetone, is not strong enough to completely elute the analytes from the column.

Table 1. Recoveries of deltamethrin and esfenvalerate in three analytical steps: bottle wash, eluate from solid-phase extraction and flow-through. The amount left in the sorbent (ENV+) was calculated by subtraction of the sum of the recovery from the three steps from 100%.

| Analytical step (number of replicates) | esfenvalerate recovery% (std dev) | deltamethrin recovery% (std dev) | determination method |
|---|---|---|---|
| 1. bottle wash (n=4) | 4 (5) | 4 (5) | measured |
| 2. flow-through (n=6) | 9 (2) | 9 (2) | measured |
| 3. eluate (n=6) | 37 (13)* | 39 (13)* | measured |
| total, step 1-3 | 49 | 49 | calculated |
| left in sorbent (n=6) | 51 | 51 | calculated |

*Results from solid-phase extractions were this time not corrected for ethion since the recovery from the different fractions should be comparable.

Recoveries when using activated and non-activated ENV+ were similar (table 2). A t-test ($p<0.05$) showed that there were no significant difference between the activated ENV+ and the non-activated. That indicates that activation is not necessary to achieve a better recovery.

Table 2. Recoveries of esfenvalerate and deltamethrin from solid-phase extraction using activated and non-activated ENV+ as sorbent and ethyl acetat/aceton 1:1 as eluent.

| Analyte (number of replicates) | activated ENV+ recovery% (std dev) | non-activated ENV+ recovery% (std dev) |
|---|---|---|
| esfenvalerate (n=3) | 51 (7) | 56 (11) |
| deltamethrin (n=3) | 54 (8) | 59 (12) |

This result is probably due to the large specific surface area of ENV+ that ensures an adequate exchange between the sample and sorbent, with or without activation. If activation were necessary, it would have to be carried out in field since the sorbent should not dry between activation and sampling. The possibility to use non-activated ENV+ is an advantage in an in field situation since the sampling method should be as simple to use as possible.

### *3.3.2. Experiment 1b*

In experiment 1b the stronger solvents ethyl acetate and dichloromethane were tested as eluents in an attempt to improve the recovery (table 3). The result shows that dichloromethane gives the highest recovery and it was therefore selected to be used as eluent in the following work. EPA (US Environmental Protection Agency) recommends that recoveries are between 70% and 130% (Tríska, 1995). Ethyl acetate is therefore also an acceptable eluent since recoveries exceeded 70%. Ethyl acetate is a better eluent from an environmental point of view and if the method should be used on a routine basis it may therefore be a better choice.

Table 3. Recoveries of esfenvalerate and deltamethrin using ethyl acetate/acetone (1:1, v/v), ethyl acetate and dichloromethane as eluents and ENV+ as sorbent in the solid-phase extraction procedure.

| experiment (number of replicates) | eluent | esfenvalerate recovery % (std dev) | deltamethrin recovery % (std dev) |
|---|---|---|---|
| 1a (n=6) | ethyl acetate/acetone (1:1, v/v) | 53 (9) | 56 (9) |
| 1b (n=6) | ethyl acetate | 75 (6) | 80 (6) |
| 1b (n=5) | dichloromethane | 93 (5) | 98 (5) |

During experiment 1b two different elution techniques were tested: direct elution and a soaking step before elution (table 4).

Table 4. Recoveries of esfenvalerate and deltamethrin using two different elution techniques: direct elution and a soaking step before elution. ENV+ was used as sorbent and ethyl acetate and dichloromethane as eluents.

| Eluent (number of replicates) | direct elution recovery% (std dev) | | soaking step recovery% (std dev) | |
|---|---|---|---|---|
| | esfenvalerate | deltamethrin | esfenvalerate | deltamethrin |
| ethyl acetate ( n=3) | 73 (2) | 78 (1) | 77 (9) | 83 (8) |
| dichloromethane (n=3) | 91 (6) * | 96 (7) * | 95 (4) | 100 (4) |

*One of the samples with dichloromethane as eluent was lost during the experiment

There was no significant difference in recovery between the two methods for any of the analytes or the eluents (t-test, $p>0.05$). That implies that the soaking step can be excluded, which is favourable since the time consumption of the solid-phase extraction procedure is decreased.

The pre-washing step decreased the amount of disturbing peaks and is therefore recommended before the use of ENV+ columns (figure 4 b and c). In studies limited to esfenvalerate and deltamethrin the pre-washing step can be excluded since no interferences appears in the area of these substances retention times (figure 4a).

As a summary of the results from experiment 1a and 1b it can be stated that a pre-washing step of the ENV+ columns are needed but that the activation step can be excluded. Dichloromethane is the best eluent and the elution can be made directly without a soaking step.

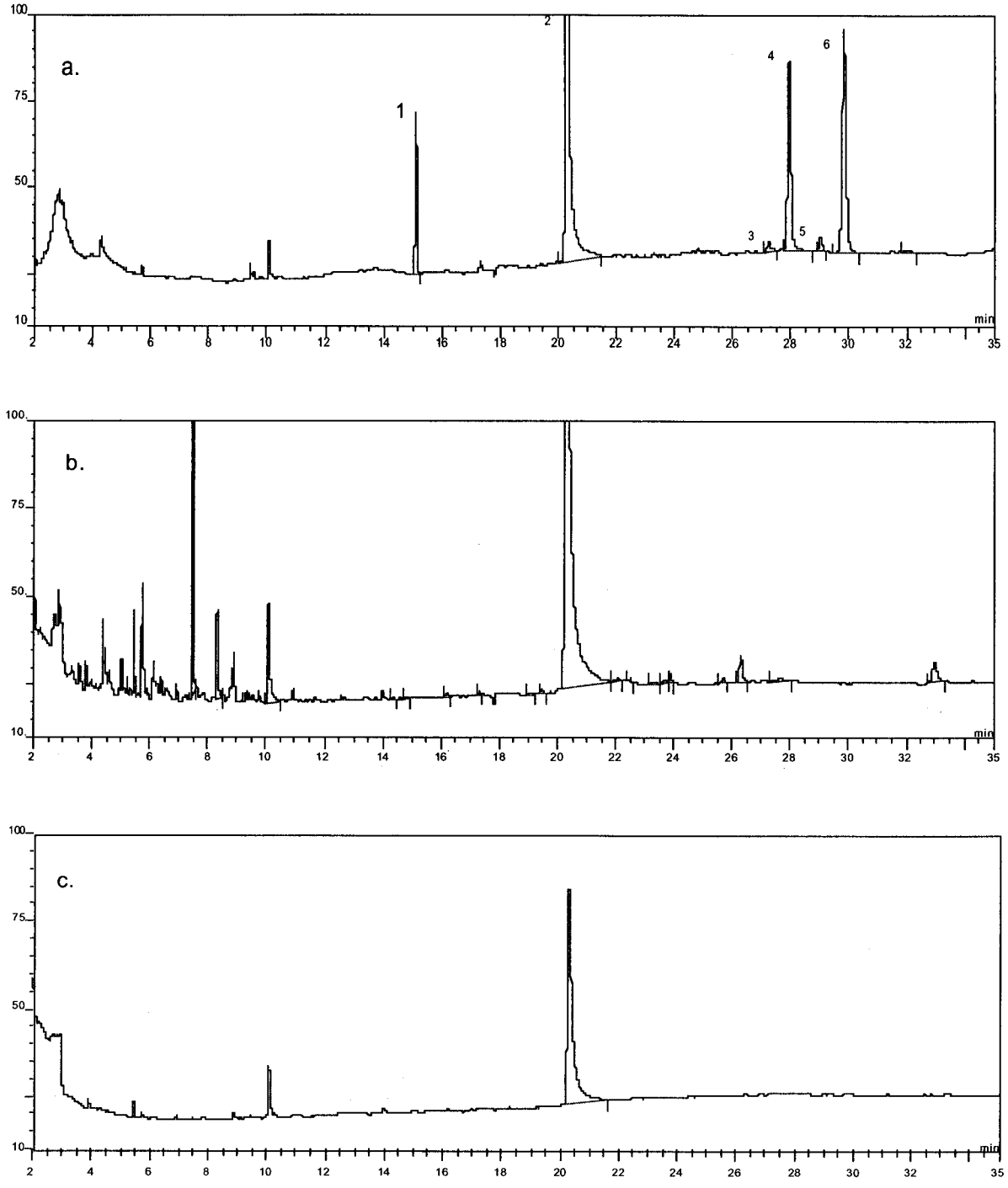



Figure 4. a) Chromatogram of a standard with internal standard ethion (1), esfenvalerate (3 and 4) and deltamethrin (5 and 6). The large peak in the middle of the chromatogram (2) is a disturbance. b) Chromatogram showing the eluate from an ENV+ column without pre-washing. c). Chromatogram showing the eluate from an ENV+ column that has been pre-washed with dichloromethane and acetone. All analysis were performed on GC-ECD.

# 4. Particle bound fraction

The aim of this experiment was to examine the possibility to determine pesticide residues adsorbed to particulate matter in surface waters.

## 4.1. Experimental

### *4.1.1. Standards and solvents*

Samples were prepared in water from river Fyris (Uppsala, Sweden) by dilution of a stock solution of deltamethrin and esfenvalerate in acetone. The final concentration of deltamethrin and esfenvalerate were 1.007 µg/l and 0.835 µg/l, respectively. Two internal standards ethion and permethrin were used. Permethrin was chosen since it has similar characteristics as the analytes and would therefore expect to behave in a similar way during extraction and final determination. Ethion was added directly to the sample and was used for evaluation of the amount of pesticides dissolved in water. Permethrin was added in the analytical step where the glass fibre filter is extracted and was used for evaluation of the amount of pesticides adsorbed to particles. Three new standard solutions with the same concentrations of esfenvalerate and deltamethrin as in the previous experiments were prepared in cyclohexane/acetone (9:1, v/v). The same amounts of permethrin and ethion were added to the standard solutions as to the samples. Dichloromethane, acetone and cyclohexane were used for the solid-phase extraction. All solvents were of pesticide grade.

### *4.1.2. Extraction equipment*

The same type of solid-phase extraction equipment was used as in the previous experiments. A filter holder with a 47 mm glass fibre filter, cut-off 0.7 µm, (Whatman GF/F, Cat No 1825 047, Whatman International Ltd, Maidstone, England) was put in front of the solid-phase column to collect particles and to prevent clogging of the sorbent. A Soxtec Avanti 2050 automatic extraction system (Foss Tecator AB, Höganäs, Sweden) and an ultra sonic bath were used for the extraction of the glass micro fibre filters.

### *4.1.3. Application of water sample*

Three spiked samples and a blank sample (water from river Fyris with the internal standard ethion) were prepared and equilibrated for approximately three, four and five hours before application. Results showed that distribution of substances between particles and water were dependent on equilibration time. New samples were therefore prepared and equilibrated for three days to achieve a stable equilibrium. Half of the samples were kept in room temperature and in darkness since deltamethrin is degraded in light (Tomlin, 1997). The other samples were stored in the dark in a refrigerator at 6 °C. The reason for this was to study the degradation of the analytes in water when stored at different conditions. Tap water samples with deltamethrin and esfenvalerate were also prepared and left at room temperature in protection from light for three days. The samples were then led through the glass fibre filter and the ENV+ column at a flow rate of approximately 35 ml/min. Each sample had a volume of 500 ml

since larger volumes tended to clog the glass micro fibre filter and thereby made the application procedure too time consuming. An unused filter was extracted with dichloromethane to check whether any interfering substances were released from the material.

*4.1.4. Solid-phase extraction*

The solid-phase extraction was performed as previously. The sample bottle was rinsed twice with 10 ml of dichloromethane and these portions were filtered through a filter with sodium sulphate and added to the eluate from the solid-phase extraction. The volume was adjusted to 1 ml at the end of procedure.

*4.1.5. Extraction of filter*

Two different extraction methods for determination of particle bound fraction were tested: ultra sonic bath with dichloromethane and Soxtec extraction with dichloromethane/acetone (1:1, v/v).

4.1.5.1. Ultra sonic bath

The glass micro fibre filters were extracted with 10 ml of dichloromethane in an ultrasonic bath for ten minutes. Permethrin was added to the solvent as an internal standard. The solvent was decanted and filtered through a filter with sodium sulphate. The extraction procedure was repeated three times. Cyclohexane (5 ml) was then added and the extract was concentrated using a rotary vacuum evaporator. The volume was adjusted to 1 ml with cyclohexane/acetone (9:1).

4.1.5.2. Soxtec extraction

Glass micro fibre filters were placed in pre-washed cellulose thimbles and permethrin was added as an internal standard. The pre-washing was carried out by running empty thimbles in the same extraction program and with the same type of solvent as for the samples. A mixture of dichloromethane and acetone (1:1, v/v) was used as solvent and the extraction was carried out in a two step procedure using a Soxtec Avanti 2050 automatic extraction system. First the sample was immersed in the boiling solvent to dissolve most of the soluble material (2h, 170 °C). In the second step the sample was raised above the solvent surface to permit efficient washing with the solvent from the condensers (1h, 170° C). After the extraction residues of solvent were collected from the condenser valves (2 min). The extract was then concentrated by evaporation for 2 minutes. The remaining extract was dried through a filter with sodium sulphate. Cyclohexane (5 ml) was added and the extract was concentrated using a rotary vacuum evaporator. The volume was adjusted to 1 ml with cyclohexane/acetone (9:1).

*4.1.6. Instrumental and chromatographic conditions*

The same instrument and chromatographic conditions were used as in experiment 1. Results were evaluated from calibration curves where analyte concentration was a function of the response of standards divided by the response of the internal standard.

## 4.2. Results and discussion

The extraction of an unused filter showed that few disturbing peaks appear from the filter material and no pre-washing before use is therefore needed (figure 5).

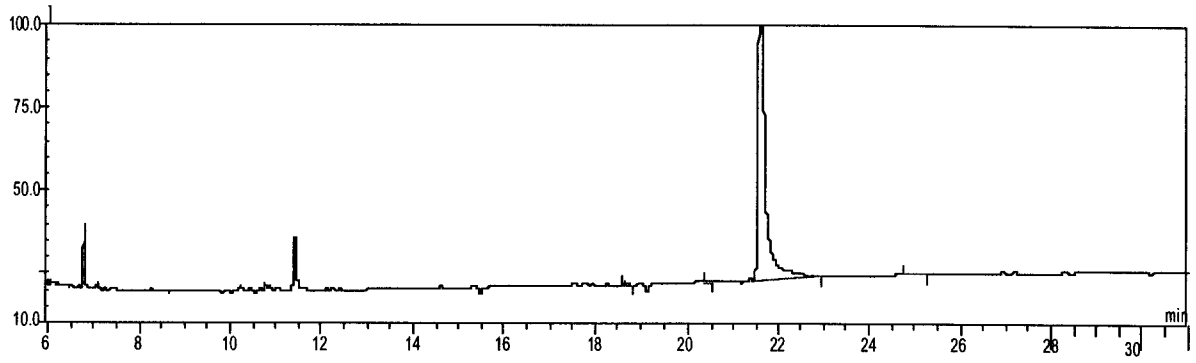



Figure 5. Chromatogram showing an extract from a glass fibre filter extracted in ultrasonic bath with dichloromethane. The extract was analysed on GC-ECD.

When using ultra sonic bath for estimation of the amount of esfenvalerate and deltamethrin in particle bound phase recoveries were low. Approximately 9% of the two pyrethroids were recovered from the particles while approximately 30% were found in the water (table 5).

Table 5. Recoveries of esfenvalerate and deltamethrin in spiked river water samples stored in refrigerator and room temperature. ENV+ was used as sorbent and dichloromethane as eluent for the water soluble fraction, and the particle bound fraction was determined by extraction of the filter in an ultra sonic bath with dichloromethane.

| fraction | storing (number of replicates) | esfenvalerate recovery % (std dev) | deltamethrin recovery % (std dev) |
|---|---|---|---|
| water soluble | refrigerator (n=3) | 29 (4) | 29 (5) |
| | room temperature (n=3) | 31 (4) | 32 (4) |
| | total (n=6) | 30 (6) | 31 (7) |
| | | | |
| particle bound | refrigerator (n=3) | 9 (1) | 9 (1) |
| | room temperature (n=3) | 10 (2) | 10 (1) |
| | total (n=6) | 9 (1) | 9 (1) |
| | | | |
| sum of the two fractions | | 39 | 40 |

There was no difference in recoveries (t-test, $p>0.05$) between samples stored in room temperature and those stored in the refrigerator (table 5). That implies that the degradation of esfenvalerate and deltamethrin by microorganisms is negligible under the time-span studied and does not affect the recoveries. Otherwise there would probably have been a larger recovery from the samples stored in refrigerator because the activity of microorganisms decreases at a low temperature. Since pyrethroids have high affinity for particles the extraction of the filters in an ultra sonic bath is probably insufficient. That hypothesis is supported by the fact that the recovery of deltamethrin and esfenvalerate from the spiked tap water samples

were 101% and 98% respectively. Tap water lacks particles and when the extraction of filters are excluded the two pesticides showed complete recovery. The low recoveries by extraction in ultra sonic bath could be due to that the filters are soaked in water and since dichloromethane and water are immiscible there might be some problems for the solvent to reach the pesticides inside the filter.

Recoveries of particle bound esfenvalerate and deltamethrin were significantly higher using Soxtec extraction (table 6). The total recovery of esfenvalerate and deltamethrin were 86 % and 80 % respectively. The Soxtec extraction seem to bee a sufficient extraction method for releasing the particle bound fraction of esfenvalerate and deltamethrin.

Table 6. Recoveries of esfenvalerate and deltamethrin in spiked river water samples using ENV+ as sorbent and dichloromethane as eluent for the water soluble fraction and Soxtec extraction of filter with dichloromethane/acetone (1:1, v/v) for the bound fraction.

| fraction (number of replicates) | esfenvalerate recovery % (std dev) | deltamethrin recovery % (std dev) |
|---|---|---|
| water soluble (n=3) | 28 (3) | 31 (3) |
| particle bound (n=3) | 58 (10) | 49 (9) |
| total | 86 | 80 |

Since different solvents were used in the two methods for extraction of filters the experiments were repeated with the same type of solvent. The mixture of dichloromethane and acetone (1:1, v/v) seemed to be the most efficient solvent and was therefore used. When an ultra sonic bath was used the filter was extracted with pure acetone for a few minutes before the addition of dichloromethane. The reason for that was to increase the efficiency of the extraction. Acetone is miscible with water and can more readily penetrate the wet filter and the collected particles, compared to the less polar solvent dichloromethane. Results from extraction of filter in ultra sonic bath and Soxtec extraction can be seen in table 7 and table 8, respectively.

Table 7. Recoveries of esfenvalerate and deltamethrin in spiked river water samples with extraction of the filters in an ultra sonic bath with dichloromethane/acetone (1:1, v/v) for the particle bound fraction.

| fraction (number of replicates) | esfenvalerate recovery % (std dev) | deltamethrin recovery % (std dev) |
|---|---|---|
| water soluble (n=3) | 42 (7) | 44 (7) |
| particle bound (n=3) | 39 (18) | 32 (17) |
| total | 81 | 76 |

Table 8. Recoveries of esfenvalerate and deltamethrin in spiked river water samples using Soxtec extraction of the filters with dichloromethane/acetone (1:1, v/v) for the particle bound fraction.

| fraction (number of replicates) | esfenvalerate recovery % (std dev) | deltamethrin recovery % (std dev) |
|---|---|---|
| water soluble (n=3) | 37 (7) | 39 (3) |
| particle bound (n=3) | 63 (10) | 52 (13) |
| total | 100 | 91 |

Soxtec extraction was still the most efficient extraction method but using ultra sonic bath with a mixture of dichloromethane and acetone gave a higher recovery than pure dichloromethane. Ultra sonic bath with dichloromethane/acetone (1:1, v/v) is an acceptable alternative extraction method for determination of the particle bound fraction, which is favourable since the Soxtec equipment is expensive and might not always be available. The results from the ultra sonic bath have a larger variation compared to results from Soxtec which implies that the former method is more inaccurate. The differences between the two Soxtec extraction batches (table 6 and 8) may be explained by the fact that there were different waters used in the two experiments. The amount and character of particles will affect the amount of pesticides adsorbed to and extracted from the solid matter.

# 5. Application of extraction methods to pesticides of different character

The aim of this experiment was to examine the possibility to use the selected extraction methods for the dissolved and particle bound fractions for a larger number of pesticides of different character. The following test substances were chosen for the experiment: atrazine, deltamethrin, diflufenican, diuron, esfenvalerate, ethofumesate, fenopropimorph, hexazinone, isoproturon, lambda-cyhalotrin, metamitron, metazachlor, pirimicarb, propiconazole, simazine and terbuthylazine.

## 5.1. Experimental

### *5.1.1. Standards and solvents*

A mixture of the sixteen test substances in acetone were prepared from stock solutions (table 9). All pesticides was provided by Dr. Ehrenstorfer Gmbh, Augsburg, Germany. The mixture of pesticides was used for preparation of spiked water samples by additions of 200 µl to river water samples from river Fyris (Uppsala, Sweden). Ethion was used as internal standard for the dissolved fraction while permethrin was used for the particle bound fraction. Three samples with a final volume of 500 ml, 500 ml and 406 ml were prepared. Samples were equilibrated for 20 h before extraction. Three external standards were prepared from the pesticide mixture by diluting it by a factor 10, 20 and 40 with cyclohexane/aceton (9:1). The two internal standards were also added.

### *5.1.2. Extractions*

The two previously selected extraction methods for quantitative determination of pesticides in the dissolved fraction (SPE using ENV+ as sorbent and dichloromethane as eluent) and particle bound fraction (Soxtec extraction with dichloromethane/acetone as solvent) were used. The pre-washing step of the solid-phase extraction columns was included since the material might release substances that can disturb the analysis.

Table 9. Mixture of sixteen pesticides in acetone that was used for spiking of water samples and for preparation of standards.

| pesticide | concentration (µg/ml) |
|---|---|
| atrazine | 0.39 |
| deltamethrin | 2.4 |
| diflufenican | 2.4 |
| diuron | 0.81 |
| esfenvalerate | 3.6 |
| ethofumesate | 0.80 |
| fenpropimorph | 2.0 |
| hexazinone | 2.0 |
| isoproturon | 0.40 |
| lambda-cyhalothrin | 1.6 |
| metamitron | 10 |
| metazachlor | 2.0 |
| pirimicarb | 0.80 |
| propiconazole | 2.0 |
| simazine | 0.80 |
| terbutylazine | 0.79 |

*5.1.3. Instrumental and chromatographic conditions*

Deltamethrin, esfenvalerate and lambda-cyhalothrin were analysed with the same instruments and chromatographic conditions as previously. The rest of the pesticides were analysed on a Hewlett Packard model 5890 gas chromatograph attached to a mass spectrometer. The GC was equipped with a CP-sil 5 CB column with dimensions 60 m x 0.25 mm i.d. and 0.25 µm film thickness. Injector temperature was 250 °C, injection volume was 2 µl and the injection was splitless. Oven temperature was set to 90 °C for 1 min, increasing 30 °C/min to 210 °C and then 4 °C/min to 300 °C, where it was held for 8 min. The MS was a VG TRIO-1 single quadrupole mass spectrometer (Finnigan MAT, Huddinge, Sweden) and it was operated in the electron impact (EI) ionisation mode at 70 eV. Data were collected in the selected ion monitoring (SIM) mode with two or three ions monitored per compound.

## 5.2. Results and discussion

The results show that the extraction methods for quantitative determination of pesticides in dissolved and particle bound fraction were applicable to a number of pesticides of different character. According to US Environmental Protection Agency the sum of the recovery of the two fractions should be between 70 and 130% to be accepted as an adequate analysis method for a certain pesticide. In this case all but three pesticides were within these limits. The recovery of terbutylazine exceeded 130% while the recovery of fenpropimorph and metamitron were lower than 70% (figure 6). The three pyrethroids, esfenvalerate, deltamethrin and lambda-cyhalothrin, were mainly found on particles which was expected due to their hydrophobic character. A small portion of diuron, fenpropimoph and hexazinon were also found on particles while the rest of the pesticides only were recovered from the water phase.

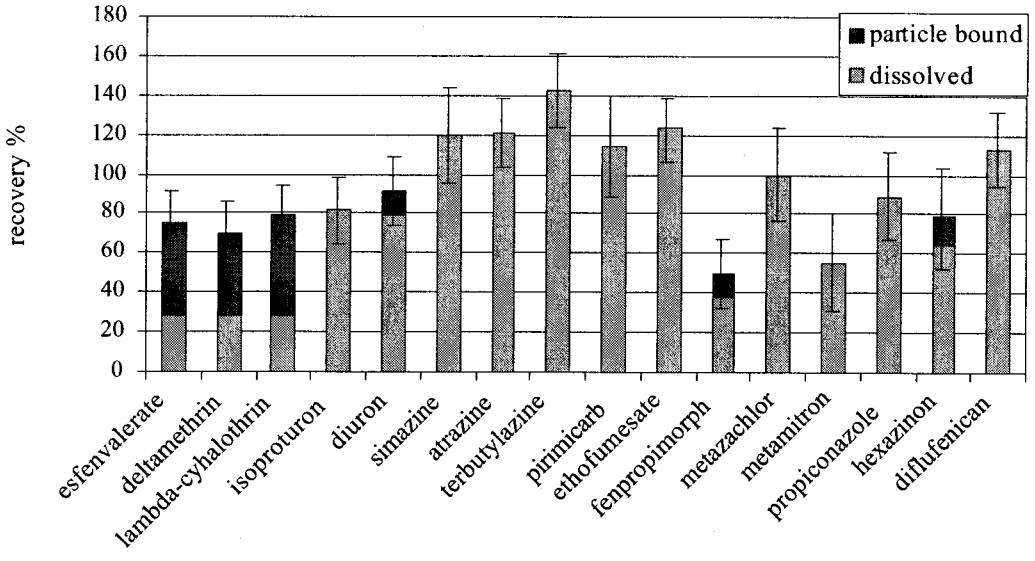



Figure 6. Total recovery and distribution between particle bound and dissolved fractions of 16 pesticides of different character (n=3).

# 6. Sampling equipment and in field study

## 6.1. Sampling equipment

The sampling equipment was constructed by connecting the filter holder and the solid-phase column with a vacuum flask and a pump (figure 7). The filter holder and all connections were made of teflon to avoid adsorption of pesticides to these parts and to reduce the amount of disturbing substances from the material (Henrik Kylin, personal communication). The filter holder was placed on a raft of foamed polystyrene to keep it floating on the water surface. A five metres long teflon tube was connected to the filter holder and the solid-phase column to make it possible to take water samples out from shore. A rebuilt bicycle pump with reversed function was used to evacuate the system. The vacuum flask was used to collect and measure the volume of the water after passage through filter and column.

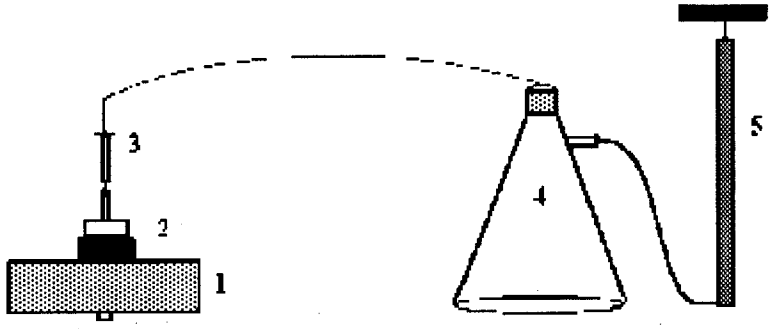



Figure 7. Equipment for sampling of pesticides in water. The sampling equipment is assembled by the following components: 1) raft, 2) filter holder with glass micro fibre filter, 3) ENV+ solid-phase column, 4) vacuum flask, 5) bicycle pump with reversed function.

## 6.2. Field study

Before sampling a few preparations had to be carried out in the laboratory. The ENV+ columns were pre-washed before use, according to the washing procedure that was described previously. They were also loaded with internal standard by letting 50 ml of ultra pure water containing 0.166 µg of ethion to flow through the columns. The columns were finally dried and were then kept wrapped in aluminium foil until sampling. The sampling was carried out by placing a glass fibre filter in the filter holder and then connecting a pre-washed ENV+ column. When the vacuum flask and the pump was attached a reduced pressure was achieved by pumping. Samples were taken in 16 ponds in intensively cultivated areas in Skåne in the most southern part of Sweden.

## 6.3. Results and discussion

The test of the sampling equipment in the field was successful. Samples from all 16 ponds were collected. The flow rate through the sampling equipment was dependent on the amount of suspended particles in the water and sometimes it was necessary to change filters during

sampling since particles tended to clog the filters. The sampling volume had to be at least 500 ml to make it possible to detect small amounts of pesticides. That volume was usually achieved within 30 minutes sampling. The pre-loading of the columns with internal standard needs further attention. The mean recovery of ethion in the 16 samples was 44% and the standard deviation was 21%. The low recovery could be due to degradation of ethion during the time between loading and sampling. Another possibility is that ethion had adsorbed that hard to the sorbent that some of it was left on the column after extraction. An alternative application procedure where the internal standard is added after the sampling could be tested to reduce the risk of degradation and too strong adsorption. A problem with that procedure is that the internal standard will not compensate for losses of analytes during sampling. Other possible explanations for the low recovery are that the internal standard was not 100% adsorbed during the pre-loading or that ethion passed through the column during sampling. Other substances that adsorb harder to the sorbent, such as δ(delta)-HCH, could be tested as internal standard to reduce losses during loading and sampling. The results with regard to pesticide residues in the ponds sampled will be given elsewhere.

## 7. Conclusions

After washing of the sorbent, ENV+, the equipment can easily be brought out in the field and sampling of a volume of 500-1000 ml can be carried out within 30 minutes. Dissolved and particle bound fractions can be separated in the field using a glass micro fibre filter in front of the solid-phase column. Extraction of the solid-phase column gives the water soluble fraction and the most efficient eluent is dichloromethane. The particle bound fraction is determined by extraction of the glass micro fibre filter. An efficient extraction method is Soxtec extraction with dichloromethane/acetone (1:1, v/v) as solvent. The described methods are applicable to a number of pesticides of different character. The greatest advantage of the solid-phase sampling method is for hydrophobic pesticides such as pyrethroids. When water samples are collected in glass bottles hydrophobic pesticides may be adsorbed to the glass of the sample bottle which leads to losses of the analytes. Since the solid-phase sampling method also facilitate transport of samples to the laboratory the technique may be useful for other kinds of organic substances. In future investigations more effort must be spent on the performance of the internal standard. Alternative application techniques or substances that bind harder to the sorbent might be among the solutions.

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