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Entwicklung, Optimierung und Validierung eines Summenparameters für potenziell bioakkumulierbare Stoffe (PBS) im Abwasser

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1 Zusammenfassung

Es wurde ein Summenparameter für potenziell bioakkumulierbare Stoffe (PBS) im Abwasser entwickelt. Er soll als Ergänzung zu den etablierten Summenparametern das Instrumentarium zur Kontrolle von (industriellen) Abwassereinleitungen vervollständigen. Die Methode zur Bestimmung der potenziell bioakkumulierbaren Stoffe (PBS) ist wie fast alle Summenparameter für organische Wasserinhaltsstoffe eine analytische Konvention.

Der Octanol-Wasser-Verteilungskoeffizient P_{OW} diente als theoretischer Ausgangspunkt für die praktische Verfahrensentwicklung. Bei der Chemikalienbewertung gilt nämlich ein Stoff dann als potenziell bioakkumulierbar, wenn dessen log P_{OW}-Wert größer als drei ist. Die Abwasserinhaltsstoffe werden über eine semipräparative HPLC-Säule nach der Größe ihrer log P_{OW}-Werte aufgetrennt und die potenziell bioakkumulierbaren Stoffe werden als Fraktion innerhalb eines definierten log P_{OW}-Bereichs („Lipophiliefenster“) aufgefangen. Dieses Lipophiliefenster wird in einem der eigentlichen Analyse vorausgehenden Analysenlauf durch die Retentionszeiten zweier Markerverbindungen ($3 < \log P_{OW} < 8$, UV-Detektion) festgelegt. Huminstoffe und andere biogene, hydrophile Wasserinhaltsstoffe werden bei dem Verfahren spätestens bei der HPLC-Fraktionierung abgetrennt. Die PBS-Fraktion wird nach dem weitgehenden Entfernen des Fließmittels (Rotationsverdampfer) bis zur Gewichtskonstanz getrocknet und die Masse an schwerflüchtigen PBS mithilfe einer Semimikrowaage (Ablesbarkeit: 10 µg) bestimmt. Um zwischen dem gelösten PBS und den an feste Abwasserinhaltstoffe sorbierten potenziell bioakkumulierbaren Stoffen differenzieren zu können, werden diese getrennt analysiert.

Die PBS werden deshalb gravimetrisch bestimmt, weil sich keiner der üblichen HPLC- bzw. GC-Detektoren, auch nicht der nach der Theorie massenproportional arbeitende Lichtstreudetektor (ELSD), für die Detektion der potenziell bioakkumulierbaren Stoffe als geeignet herausstellte. Da bei einer einfachen Wägung die Masse direkt bestimmt wird, wird jede Substanz unabhängig von ihren physikalisch-chemischen Eigenschaften, a priori mit der gleichen Empfindlichkeit erfasst. Zudem wird die Gravimetrie der Forderung nach DIN 38 402 Teil 1 gerecht, dass Gehalte an Wasserinhaltsstoffen wenn

möglich als Massenkonzentrationen angegeben werden sollten. Ein weiterer Vorzug ist, dass die Analyten bei der Quantifizierung nicht zerstört werden und somit für weitere Untersuchungen, z. B. einer Einzelstoffidentifizierung oder der Bestimmung des Kohlenstoff- oder AOX-Gehaltes, zur Verfügung stehen.

Bei der Festphasenanreicherung und der semipräparativen HPLC traten durch „Bluten" der stationären Phasen Blindwerte bis maximal 160 µg (über das Gesamtverfahren) auf, sodass eine Bestimmung von PBS-Konzentrationen ab ca. 0,5 mg/l als sinnvoll erscheint (3-facher Blindwert). Über eine Auswahl anderer Markerverbindungen kann das Fraktionierungsfenster schnell und einfach neu definiert werden. Damit wird eine hohe Flexibilität des Verfahrens erreicht. Somit kann man beispielsweise auf (gesetzliche) Änderungen bei der Chemikalienbewertung unmittelbar reagieren und das Verfahren auf die neue Problemstellung zuschneiden.

Das mit Standardverbindungen erarbeitete Verfahren wurde für die Bestimmung von PBS in Abwasserproben von Kläranlagenabläufen angewendet. Kommunale und industrielle Kläranlagen verschiedener Branchen wurden beprobt (2 h- bzw. 24 h-Mischproben). Die PBS-Konzentrationen in den beprobten deutschen Kläranlagen (kommunal und industriell) waren in der Regel niedrig (< 1 mg/l). Erhöhte PBS-Gehalte wurden bei einem Abwasser eines Indirekteinleiters aus der metallverarbeitenden Industrie erhalten (> 1mg/l). Zusammenhänge zwischen dem PBS und den Summenparametern TOC, DOC und AOX wurden, wie zu erwarten war, nicht festgestellt. Zudem waren keine Korrelationen zwischen den PBS-Konzentrationen und der Größe, der technischen Ausstattung, der örtlichen Lage und der Art des Abwassers feststellbar.

Im Rahmen einer Kooperation mit dem niederländischen RIZA wurde eine vergleichende Untersuchung mit dem an dem jeweiligen Institut entwickelten Verfahren durchgeführt. Das niederländische SPME-GC/MS-Verfahren und die gravimetrische PBS-Methode wurde an sieben industriellen Abwässern verschiedenener Branchen angewendet. Dazu wurden Stichproben aus den Abläufen der Kläranlagen (Direkteinleiter) genommen.

Die PBS-Konzentrationen dieser industriellen Abwasserproben lagen in einigen Fällen deutlich über denen, die bei den deutschen Abwässern erhalten wurden. Auch wurden zum Teil erhebliche Unterschiede zwischen dem gelösten und dem feststoffgebundenen PBS gefunden. Die PBS lagen in zwei Fällen fast vollständig (über 90 %) an Feststoffe adsorbiert vor. Auch die TOC- bzw. DOC- und AOX-Gehalte der niederländischen Abwasserproben waren im Vergleich zu den deutschen Abwasserproben zum Teil deutlich höher.

Ein Vergleich der mit den beiden Verfahren (SPME/GC-MS bzw. SPE/Gravimetrie) gefundenen Analysenwerte zeigte gewisse Tendenzen auf, obwohl keine direkten Zusammenhänge ableitbar waren. Die Gründe hierfür liegen in der prinzipiellen unterschiedlichen Vorgehensweise bei den beiden Verfahren. Während die potenziell bioakkumulierbaren Stoffe bei dem niederländischen Verfahren passiv angereichert werden, werden sie bei der deutschen Methode erschöpfend extrahiert. Bei dem gravimetrischen Verfahren werden leichtflüchtige PBS nicht erfasst. Mit der GC-MS-Methode hingegen werden nur GC-gängige Verbindungen und Substanzen detektiert, die underivatisiert ionisierbar sind, während bei der Gravimetrie diese Limitierungen nicht existieren.

Die Analysenergebnisse im Rahmen dieser orientierenden Erkundungen zeigten, dass der Summenparameter PBS eine sinnvolle Ergänzung zu den Summenparametern für organische Wasserinhaltsstoffe darstellen kann. Primäre Aufgabe in der Zukunft wird vor allem sein, die Anwendbarkeit der Methode im Routinebetrieb zu ermitteln.

2 Summary

2.1 Introduction

One of the main results of the 4^{th} *International North Sea Conference* was the decision to reduce the emissions, discharges and losses of hazardous substances in order to prevent toxic effects to marine organisms. In accordance with this general aim, the objective of the *OSPAR-Commission with Regard to Hazardous Substances* was to prevent pollution of the maritime area by continuously reducing discharges, emissions and losses of persistent, toxic and bioaccumulating substances, with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances. The OSPAR-Commission will develop programmes and measures to identify, prioritise, monitor and control the emissions, discharges and losses of hazardous substances. So far no method exists which allows the determination of potentially bioaccumulating substances in water samples. For this purpose, in this research project a summative parameter for the determination of potentially bioaccumulative substances (PBS) in effluents of waste water treatment plants has been developed.

The distribution of an organic compound in the solvent-system octanol-water is the simpliest approach to describe the behaviour of bioaccumulation in organisms. It has been often reported that there are close correlations between log P_{OW} and log BCF values for different classes of organic compounds. It has been also demonstrated that the n-octanol/water partition coefficients of organic compounds can be detected by reversed-phase HPLC, because good correlations between log P_{OW} values and the logarithm of the capacity factors (log k’) obtained by RP-HPLC have been found. With respect to these relationships, a HPLC-method has been developed for the quantification of potentially bioaccumulating substances. It was recommended by the Technical Guidance Document (TGD) that all organic compounds with log P_{OW} higher than three should be regarded as potentially bioaccumulative.

Traditionally, regulatory approaches to assess the bioaccumulation potential of hydrophobic organic chemicals have emphasized the direct accumulation from solution, leading to the development of determination methods of the bioconcentration factor (BCF) as a measure of direct uptake of dissolved substances. However, often a large fraction of the lipophilic compounds in water is not dissolved, but is partitioned among suspended sediments and particulate matter. Non-polar organic micropollutants adsorbed to sediments are normally less bioavailable to freshwater and marine animals compared to freely dissolved contaminants. But benthic organisms ingest the contaminated particulate matter, and the adsorbed compounds get bioavailable. Moreover, this can lead to a food chain accumulation. In our opinion, it is therefore imparative also to determine the PBS adsorbed to suspended solids.

2.2 Aim

The summary parameter PBS should comply with several prerequisites:

1. **All** relevant organic compounds with a tendency for bioaccumulation should be detectable by the method.
2. The response factors and the recoveries of all unknown analytes should be equal.
3. Humic acids should be separated from the PBS.
4. In order to determine all PBS, an exhaustive extraction method should be used.
5. The PBS-determination should be applicable both to the liquid phase and to the PBS adsorbed to suspended solids, because solid-bounded potentially bioaccumulative substances are regarded as a hazard criterion for benthic ecosystems, especially for the marine environment.

2.3 Experimental

The crucial analytical step of the developed method is the separation of the PBS by RP-HPLC according to their lipophilic character and their tendency for bioaccumulation. Not all of the potentially bioaccumulative substances present in a sample are normally known. They may differ totally in their structural and chemical properties. Therefore, the detection method should be universal for all compounds contributing to PBS and the response factors should be equal for all (unknown) organic substances. In view of their utilizability for PBS determination, several detectors were investigated: GC-FID, GC-ECD, HPLC-UV/VIS and MSD coupled with GC and HPLC. The evaporative light scattering detector (ELSD) is described in literature as a mass proportional detector. Therefore, this detector was tested too. None of the GC- and HPLC-detectors has shown a mass proportional response for the numerous standard compounds tested and therefore none of them were applicable in this method.

Our investigations demonstrated, that gravimetric detection is a practicable alternative. To reach a detectable mass level of PBS, the upscaling of the method to semi-preparative dimensions was necessary. An advantage of this direct mass detection is that it is a non-distructive method. This allows further analyses of the PBS sample. Because the unit of the PBS is mg/l, it is possible to directly compare PBS with other conventional summary parameters, such as DOC, TOC and AOX.

2.4 Procedure

In the present research project the following method for the determination of PBS in waste water has been developed (Abb. 1). 1 l of the waste water sample is filtered by a glass fibre paper. The filter is washed three times with 10 ml of distilled water. The filter is dried in a circulating air drying oven at 30 °C and then it is placed in a desiccator until weight constance.

The mass of the filter is calculated as:

$$m_{AS} = \frac{m_T}{V_P} \cdot f \qquad m_T = m_V - m_L \quad \text{(Gl. 1)}$$

m_{AS}	mass of filterable substances in mg/l
m_T	dry mass in g
m_V	mass of filter after filtration in g
m_L	mass of filter before filtration in g
V_P	sample volume in l
f	factor (f = 1 000)

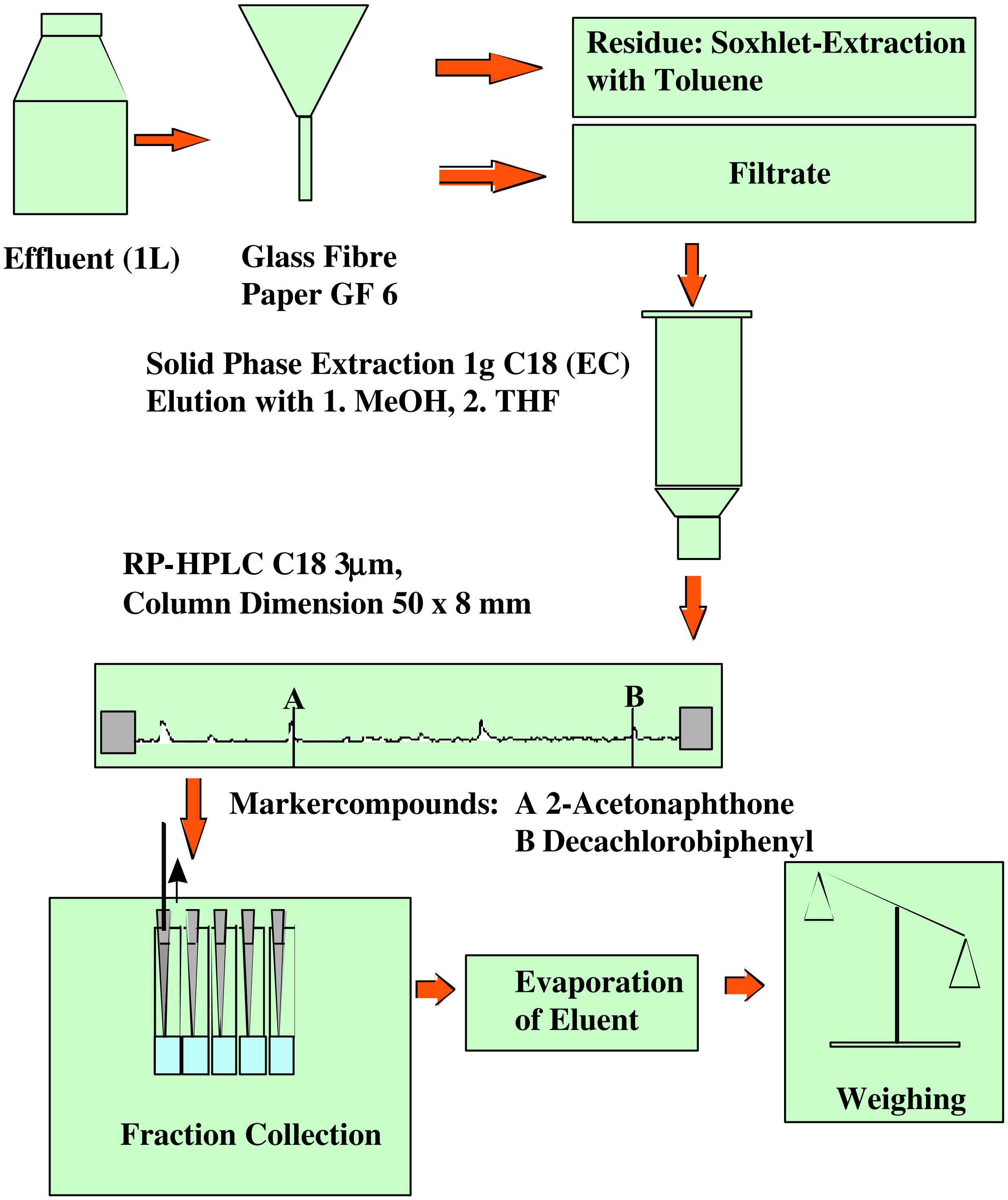

Abb. 1: Scheme of the method

After drying, the filter is extracted by toluene soxhlet extraction and the filtrate is extracted by solid phase extraction (SPE). With a SPE vacuum chamber, it is possible to extract 12 samples in parallel.

The SPE-column (1 g of RP-C18, endcapped) is dried in gentle stream of nitrogen. The adsorbed compounds are successively eluted with two column volumes of methanol and two column volumes tetrahydrofuran from the solid phase. The eluates are confined with a speed vac (50 °C, 60 mbar) to 1,5 - 2 ml. The extracts were injected onto a semipreparative RP-HPLC-column (C18, 3 µm, 50 x 8 mm). The mobile phase was acetonitril/water (5 mmol/l ammonium acetate buffer). The mobile-phase flow rate was 3.2 ml/min. After each analysis the column is washed with tetrahydrofuran in order to reduce the blank.

The following gradient program was applied:

Time in min	H_2O/NH_4CH_3COO (5 mmol/l) in %	ACN in %	THF in %
0	70	30	0
2	60	40	0
5	50	50	0
8	20	80	0
10	0	100	0
20	0	100	0
25	0	0	100
45	0	0	100
50	0	100	0
55	0	100	0
60	70	30	0

In a separate HPLC run, the retention times of two marker compounds define a "lipophilicity window". The used marker compounds were 2-acetonaphthone (log $P_{OW} = 2{,}85$) or bromobenzene (log $P_{OW} = 2{,}99$) and decachlorobiphenyl (log $P_{OW} = 8{,}27$). The fraction within this window (PBS-fraction) is collected. The solvent is confined with a rotary evaporator (50 mbar, 60 °C) and transfered into a porcelain crucible (for further TOC analysis) or an aluminium vessel. The PBS-fraction is lyophilized with a freeze drying apparatus (-10 °C, 0,220 mbar) or dried in a circulating air drying oven at 30 °C. The dried PBS is placed into an desiccator until the weight is constant. Finally, the PBS is quantified gravimetrically with a semi micro balance. The TOC of the PBS can be determined with a multi N-C-analysator.

The concentration oft the potentially bioaccumulative substances PBS is calculated from:

$$c\,(\mathrm{PBS}) = (m_v - m_l) \cdot \frac{V_{ges}}{V_{inj}} \cdot \frac{1}{V_P} \quad (\mathrm{Gl.}\ 2)$$

c (PBS)	PBS concentration in g/l
m_v	mass of the sample vessel after drying in g
m_l	mass of the empty sample vessel in g
V_{ges}	volume of the eluate in ml
V_{inj}	volume of the sample loop in ml
V_P	volume of the extracted waste water sample in l

2.5 Results and Discussion

The developed summary parameter PBS has several advantages. It represents a fast procedure, no sophisticated analytical equipment is required. The determination of all potentially bioaccumulative organic compounds present in a sample is possible, largely independent of their structural and chemical properties. The method is applicable to potentially bioaccumulative substances in the liquid phase and PBS adsorbed to suspended solids. Using the direct mass detection by weighing, there are no problems with the variation of the response factors for different class of compounds. The PBS-value has the dimension of a mass concentration and the unit is mg/l. This comfortably allows to compare the PBS with other summary parameters, such as TOC, DOC, COD, BOD or AOX. The facile change of the marker compounds gives a high flexibility by defining the “lipophilicity window”. The gravimetric detection is a non-distructiv method, so further analysis are possible. The humic acids are removed at the latest by the HPLC separation, because they are outside of the “lipophilicity window”.

The method to determine potentially bioaccumulative substances (PBS) is an analytical convention. For example, volatile PBS cannot be detected by this procedure. Furthermore, the limitations of the octanol-water model, which is normally used to describe the phenomenon of bioaccumulation, subsequently also define the limitations of the newly developed summary parameter. As an example, a functional relationship between log P_{OW} and log BCF values can only be observed, if the process of bioaccumulation is based on passive diffusion. Consequently, specific mechanisms of bioaccumulation in organisms, such as active transport mechanisms or the bioaccumulation processes of surfactants, cannot be described by this model. These organic compounds are not detectable by the summary parameter PBS, if they have a log P_{OW} lower than three.

Another limitation of log P_{OW} as screening parameter is that the log-log correlation between the bioconcentration factors in organisms and the n-octanol/water partition coefficients deviates from linearity for organic compounds with a molecular weight greater than 600 Da, because the uptake is controlled by the size of the pores in the lipid membrane. This phenomenon cannot be registered by the PBS summary parameter.

A prerequisite of the PBS summary parameter was, that the degree of recovery of all compounds within the “lipophilicity window” should be comparable. This was realized by a solid phase extraction with a fractionated elution with methanol and tetrahydrofuran. The recoveries of the standard compounds varied from 60 to 100 %.

A major problem of this detection method was the relatively high blank values, which probably are caused by “bleeding” of the used C 18-materials. The blank value was minimized to 80 to 160 µg/l by washing the stationary phases with tetrahydrofuran before use. The detection limit of PBS is 0.5 mg/l, determined by the threefold blank.

A further advantage of the new method is the possibility of changing the marker compounds, which allows a high flexibility in defining the „lipophilicity window“. In this way, it is possible to react very fast to changes in the guidelines of chemical assessment. One disadvantage of the new PBS parameter is that in semipreparative

HPLC normally broad signal peaks are obtained. A reduction of the peak width could be realized by the optimization of the gradient elution program.

The developed method was validated with several waste waters from different sewage treatment plants (STP). The samples were taken from the effluents of municipal STPs, as well as from STPs of different industrial branches (2 h or 24 h mixed samples). Biogenic water ingredients, e. g. humic substances, were selectively removed from the PBS by semipreparative HPLC. Most of the PBS, both in the liquid phase and adsorbed to suspended solids, were found in lower concentrations than 0.5 mg/l. Only two samples from indirect discharges from metal working showed higher concentrations of 1.3 and 2.7 mg/l. One effluent from a plant from pharmaceutical industry exhibited PBS of 0.7 mg/l.

Correlations between PBS and the summary parameters TOC, DOC and AOX were not found. Relationships between the PBS and the effluent composition of the STP, as well as the size of the STP, the technical equipment and the location could not be demonstrated by our investigations.

In a cooperation with the Dutch *Institute for Inland Water Management and Waste Water Treatment (RIZA)* a GC-MS-based method involving solid phase micro-extraction (SPME) was compared with the developed summary parameter PBS by the measurement of seven effluents (direct introducers) representing a wide variety of industrial branches. All effluents were random samples.

The PBS values in the liquid phase of a waste water sample of a food oil extraction factory, a pesticide producer and a metal blast furnace were lower than 0.4 mg/l, whereas the concentration in the solid phase of the respective samples of the food oil extractor and the metal smelting industry were much higher with 5.5 mg/l and 11.3 mg/l. With respect to these results, more than 90 % of PBS were bound to suspended solids. One reason for this phenomenon could be the high suspended matter content of the samples (76 mg/l for the sample from food oil extractor, 218 mg/l for the sample from metal blast furnace).

High concentrations of 0.9 mg/l and 2.2 mg/l PBS were found in the liquid phase of the sample from the effluent of a paper mill and a waste incineration facility, respectively. The highest PBS value in the liquid phase of 13.8 mg/l was detected in a waste water of a metal plating company. The corresponding PBS solid-bound was 2.2 mg/l. The C-content of the PBS, which also was determined, was in the range from 21 to 55 %. Only in the effluent of the metal plating company the C-content in the liquid phase was much higher with 90 %.

Several correlations between the SPME/GC-MS- and the HPLC/gravimetric method were found. The Dutch approach to quantify the bioaccumulative compounds is defined as the summary parameter **T**otal **T**arget **C**oncentration (TTC). In the effluents of a pesticide and a chemicals producing factory both methods showed low concentrations of PBS and TTC, respectively. The TTC and the PBS of the effluent of the metal plating factory were much higher. There was only a discrepancy between the two methods in the case of the waste water sample of the metal blast furnace, where the detected value for the TTC was low and the PBS was high. These differences can be explained by the spectrum of detectable substances, which due to the different extraction and detection methods is different for the two methods, at least in part.

In future further investigations are needed to prove the applicability of the PBS summary paramether as a routine method for surveillance of industrial discharges.