OSPAR CONVENTION FOR THE PROTECTION OF THE MARINE ENVIRONMENT OF THE NORTH-EAST ATLANTIC

MEETING OF THE OSPAR OFFSHORE INDUSTRY COMMITTEE (OIC)

PARIS: 12-16 MARCH 2007

Bioconcentration Factors of Surfactants

Presented by the Netherlands

This document presents the results of the intersessional work 2006/2007 on surfactants. (OIC 2006/07, Product 4).

Background

1 At OIC 2006 the Netherlands presented a proposal for a protocol to determine a sorption constant for surfactants (K_{oc}) as an instrument for the registration of surfactants according to the HMCS (OIC 06/2/3-E). It provided a valuable review of the current state of understanding of sorption kinetics in sediment systems and their potential application, as well as a comprehensive discussion of the problems involved in determining sorption constants for surfactants on the basis of K_{oc} or deriving them from existing data. In the report it is concluded that because of the specific behaviour of surfactants, normalisation of sorption constants to organic carbon content (K_{oc}) is therefore not recommended. The report contains a proposal to either calculate a distribution coefficient K_p, using the type and the molecular structure, for sorption or to determine K_p experimentally.

2. EOSCA welcomed the report from the Netherlands, but requested that the proposal should not be adopted in its present form until an experimental protocol that could be used for screening or as a definitive test, and properly ring-tested and evaluated, is available.

3. Spain was of the view that the EC, as Contracting Party to OSPAR, should be consulted for their views about this work.

4. The UK was of the view that Contracting Parties should continue for the time being with the application of the current OSPAR instruments system while performing in parallel a ring-test of the proposed protocol when assessing chemicals for the validation of the results.

5. After discussion, OIC agreed, *inter alia*, that both the systems proposed by EOSCA and the Netherlands should be combined. The Netherlands was invited to further develop the proposed protocol for the determination of sorption constants for surfactants for presentation at OIC 2007, taking into account the opinion of the EC and developments of the Technical Guidance Document (TGD) with regard to this work.

Results of intersessional work

6. The organic carbon adsorption coefficients K_{oc}, are a mandatory requirement for surfactants according the current Appendix 1, section 2.5 of the OSPAR Recommendation 2000/5. Since there is no agreement yet how to determine the adsorption of surfactants, the Netherlands was requested by OIC to come up with a way how to determine this. The research carried by the Netherlands for OIC 2006 was to determine a way how to establish that. As mentioned above, the outcome of the research was that the K_{oc} was not the right parameter to use, but resulted in a proposal with a protocol to use the K_p in stead of the K_{oc}.

7. However, the study also revealed that literature data on K_p of sufficient quality is very limited and that substantial work would be necessary to establish reliable sorption coefficients for surfactants. Even the use of default values for K_p within the protocol, as proposed by EOSCA, should be based on experimental data, taking the variety of surfactants into account.

8. The Netherlands consider this proposal to be of importance for the assessment of the possible impact of surfactants on marine sediments, as calculated based on the CHARM model. Up to now, due to the lack of

data on the partition of surfactants, this was not possible to do according to scheme 6, calculation equation 26b, mentioned in the CHARM Manual User Guide version 1.4, 3 February 2005.

9. During the discussion of the Dutch proposal for the protocol it is further concluded that considerable work is still necessary, including the evaluation of the potential of K_p to act as a predictor of bioaccumulation potential. This research did not result in a proposal on the way how to assess the bioaccumulation potential for surfactants.

10. The HMCS Pre-Screening usually prescribes to use the Log P_{ow} to predict the bioaccumulation potential. However, both the protocols OECD 107 and OECD 117 are not applicable for surfactants. As a consequence the PBT assessment using the HMCS Pre-Screening with regard to the bioaccumulation potential for surfactants is based on the precautionary principle. That means that if the supplier or producer does not present evidence, i.e. on a BCF study, then the surfactant is assumed to have a bioaccumulation potential. However, such an assumption may lead to the result that most of the surfactants are potential candidates for substitution (2 out of 3-PBT criteria leading to substitution in accordance to the HMCS Pre-Screening Scheme as mentioned in the Appendix of OSPAR Recommendation 2000/4).

11. Based on the above, the Netherlands is of the opinion that there should also be a way forward to establish a way on how to predict the bioaccumulation potential for surfactants. Therefore, in the follow-up study priority was given to establish reliable descriptors for bioaccumulation of surfactants. In Annex 1 of this document the outcome of the study is presented. The aim of the study was:

i. to establish a dataset of reliable BCF values for surfactants from literature data;

ii. to investigate the possible existence of quantitative relationships between the set of reliable BCF and reliable K_p values;

iii. to discuss how to proceed in case the established correlations between BCF and K_p are weak or do not exist.

12. So far the Netherlands has delivered two tools to deal with surfactants as part of the PBT assessment, i.e.

i. A proposed protocol to determine the sorption coefficient for surfactants as part of the PEC / PNEC risk approach as determined in Appendix I, Roman III paragraph 7 of the OSPAR Decision 2000/2; and

ii. A proposal to predict the bioaccumulation potential for surfactants as part of the HMCS Pre-Screening Scheme shown in Appendix 1 of the OSPAR Recommendation 2000/4.

Action requested

13. OIC 2007 is invited to:

a. examine the results on the follow-up study on surfactants;

b. discuss the possible consequences for the HMCS and CHARM;

c. agree on a possible way forward for the adoption of these two proposals for the PBT assessment of surfactants.

Bioconcentration factors

of surfactants in seawater

Draft 3

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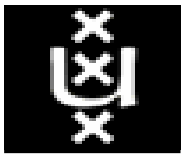
(THIS IS A DRAFT REPORT DO NOT CITE OR QUOTE)

Amsterdam, February 2007



Colophon:

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Content

Preface

In a document published by the European Oilfield Speciality Chemicals Association (EOSCA, 2000), the K_{oc} parameter has been proposed as a possible measure for the estimation of the bioaccumulation potential of surfactants, rather than octanol/water partition coefficient, K_{ow}. The latter is frequently used for the estimation of bioaccumulation potential of hydrophobic organic compounds. The same document stated that "Whether this will provide a more reliable data set upon which to base environmental risk assessments of surfactants is uncertain at present, particularly since there are currently no standardised test methods available and no inter-laboratory comparisons (ring tests) on which to formulate test validity criteria."

At the meeting of the OSPAR Offshore Industry Committee 2006 in Copenhagen, the Netherlands were invited to present at OIC 2007 a further refined proposal for a protocol to determine a sorption constant for surfactants. Taking into account the comments made at OIC 2006 and the fact that the sorption constant is a prescriptor of the bioaccumulation potential in the current evaluation procedure for surfactants used in off shore applications, priority was given to investigate literature data on (reliable predictors of) bioaccumulation of surfactants. Therefore, the North Sea Directorate of the Dutch Ministry of Transport, Public Works and Water Management committed the University of Amsterdam to report the present state-of-the-science about bioconcentration of surfactants.

The present study report reviews the current scientific knowledge about bioconcentration of surfactants and addresses the items indicated above. This report evaluates the type of interactions involved in the bioconcentration process. A method is provided to relate the BCF of a surfactant to a suitable parameter (descriptor).

Summary

In the Harmonised Offshore Chemical Notification Format the bioaccumulation potential is one of the three selection indicators of a substance. The key parameter for evaluating potential bioaccumuation and the possible additional risk of the substance in the marine environment is the octanol-water partition coefficient, K_{ow}. However, for surfactants this hydrophobic parameter cannot be determined experimentally. As a consequence, surfactants will receive automatically a negative flag, unless an experimentally determined bioconcentration factor (BCF) results in a value below 100 L/kg. In order to avoid that for each surfactant an expensive BCF experiment must be conducted EOSCA (2000) proposed to relate the bioaccumulating potential to another hydrophobic parameter, viz. the organic carbon normalised sediment-water partition constant, K_{oc}, of the surfactant. In a previous study (Krop & de Voogt 2006) sorption constants of surfactants in seawater and river water were critically evaluated. In that study it was concluded that the interaction of surfactants with marine sediments is mainly electrostatic in nature. Consequently, normalisation of sorption constants of surfactants to organic carbon is meaningless and the resulting substance parameter K_{oc} is not a constant.

In the present study the BCF values of surfactants reported in the literature were critically evaluated. First a set of useful BCFs was established to be used in correlation attempts with reliable sorption constants (non normalised), from the previous report. Second the analysis was used to deduct the driving force behind the bioaccumulation process of surfactants in seawater. Identifying the driving force would allow to derive the appropriate types of parameters for successfully predicting bioaccumulation potential.

In total 140 BCFs for anionic, 82 for cationic and 35 for nonionic surfactants were found in the scientific literature since 1994. The BCFs reported in literature prior to1994 were not considered to be useful since all were based on radioactive measurements. Such measurements would exclude a proper distinction of any biotransformation process occurring during the bioaccumulation test. After application of criteria to evaluate the quality of the data reported, from the 257 values only 41 BCFs were considered to be useful. It should be noted that for only 3 surfactants experimental data were actually determined in seawater.

The BCF depends on the species used in the experimental test. Most surfactants are metabolised by e.g. fish.. In addition the BCF varies substantially with the feeding regime applied in the tests. If the kinetic scheme according to the OECD 305 protocol for obtaining experimental BCF values is followed, the resulting BCF is systematically higher than the steady state one. The BCF decreases with increasing molecular mass of the substance and with decreasing exposure concentration. However the exact relationships are currently unknown. Owing to a lack of experimental BCF obtained in seawater the influence of the ionic strength is also unknown.

Although in chemicals policy a surfactant is often indicated as a single substance (i.e. single CAS number), surfactants applied in off shore practice are frequently composed of a mixture of different homologues. The BCF of such a mixture can be obtained by summation of the fractional BCFs of single components present in the mixture.

Current scientific literature indicates that the nature of the bioaccumulation process of surfactants is hydrophobic in character. Therefore the search for suitable hydrophobic parameters to explain the variation of BCF is still legitimate. Although different log K_{ow}

estimation methods show high correlations with BCF, EC50 or CMC values, these log K_{ow} estimation methods cannot be critically evaluated since experimental values cannot be established. Therefore other hydrophobic parameters need to be selected that can be determined easily and unequivocally. Chromatographic methods are especially useful alternatives since it is well known that the capacity factor of the substance may lead to high correlation with e.g. its K_{ow} or aqueous solubility. However the chromatographic method employed must ensure that the interaction of the surfactant with the column is hydrophobic and that the mobile phase is varied to correct to a 100% water phase. The existing OECD 117 protocol would need a minor modification to serve this purpose. After establishing a set of capacity factors as described above for a relevant group of each type of surfactant, their corresponding BCF need to be determined experimentally for a relevant marine species. These values are correlated in the same way as the log K_{ow} QSAR in the OECD 117 method. The capacity factor of the surfactant is then related to the BCF value.

1. Introduction

Chemical substances used above a certain quantity in the offshore industry are (pre-)screened for their potential environmental risks. The pre-screening is based on a PBT approach where persistency (P), bioaccumulation (B) and toxicity (T) of the pertinent substance are considered (OSPAR Recommendation 2000/4). The necessary data are submitted through a harmonized offshore substance notification system. In this harmonized system it is explicitly stated that the octanol-water partition coefficient (K_{ow}) and the sediment-water partition coefficient (K_{oc}) for surface-active substances cannot be determined.

The bioaccumulation potential of compounds is evaluated on the basis of the bioconcentration factor (BCF). The experimental determination of BCFs is a time consuming and expensive effort. For surfactants relatively few experimental BCF are available from the literature. Where experimental BCFs are lacking, estimation methods have been proposed to predict BCFs from K_{ow}. As the K_{ow} for surfactants cannot be determined experimentally, this estimation approach cannot be applied in the case of surfactants. Consequently, one of the three pre-screening parameters (B) for surfactants will be evaluated negatively. If one of the other two parameters (P or T) is also negatively evaluated the surfactant may not be released into the marine environment.

Surfactants are substances with a polar/ionic head and a hydrophobic tail. The newly developed Globally harmonized System (GHS 2003) for classification and labeling of substances and mixtures recommends, due to the variety of different head groups, to classify surfactants according to their surface activity rather than by chemical structure. The bioaccumulation potential of surfactants should thus be considered in relation to the different subclasses (anionic, cationic, non-ionic, or amphoteric) instead of to the group as a whole. This approach is followed in this report as well.

In the offshore large quantities of surfactants are used. Contrary to hydrophobic substances, a simple pre-screening of the bioaccumulating potency of a surfactant is impossible owing to their complex behaviour in the (marine) water compartment. Generally the BCF of hydrophobic substances correlates quite well with their octanol-water partition coefficients (K_{ow}). This correlation is thought to be absent in case of surfactants mainly due to the fact that the K_{ow} of surfactants is ill defined. In 2000 EOSCA (EOSCA 2000) proposed to use the sediment-water partition coefficient normalized on organic carbon, K_{oc}, of a surfactant as a descriptor for the BCF instead of the K_{ow}. In addition the K_{oc} is used as an input parameter for the CHARM fate model. This model, used in the evaluation of chemicals used in offshore industry, estimates the environmental risk of a substance in the marine compartment.

Since 2000 several initiatives have been developed initiated by EOSCA and the Dutch representation to improve the modeling of the behaviour of surfactants in the marine compartment. One of these included the consideration of other partitioning coefficients, e.g. sediment/water sorption coefficients as suitable predictors of bioaccumulation potential. To this end the sorption behaviour of surfactants has to be evaluated. At the 2006 OSPAR meeting of the OIC in Copenhagen the report entitled “Proposal for a protocol to determine sorption constants of surfactants in seawater” (Krop and de Voogt 2006) was discussed and approved. This report concluded that sorption of surfactants is non linear and mainly governed by electrostatic interactions between compound and sorbate, rather than by amount of organic carbon in the sediment. Consequently, K_p rather than organic carbon normalized sorption coefficients should be used for describing sorption behaviour of surfactants. The

report also concluded that only about 25% of the available literature K_p values for surfactants were considered of sufficient quality to be potentially useful for correlation purposes. In their comment on the proposal of the protocol EOSCA (EOSCA 2006) emphasized that, although a good start was made and several important issues were addressed, still a substantial amount of work needs to be done, including the evaluation of the potential of K_p to act as a predictor of bioaccumulation potential.

The main difficulties in the determination of the bioaccumulation potential of surfactants were documented by EOSCA in a review that was presented at Chemistry in the Oil Industry VII (McWilliams 2001). In order to establish useful descriptors for predicting the BCF through QSARs, reliable datasets of both the BCF and other descriptors like the K_p, are required.

The aims of this research project are:

- To establish a dataset of reliable BCF values for surfactants from literature data.
- To investigate the possible existence of quantitative relationships between the set of reliable BCF and reliable K_p values.
- To discuss how to proceed in case the established correlations between BCF and K_p are weak or do not exist

Reading pointer

In **Chapter II** we discuss the several ways BCFs can be determined and estimated. In **Chapter III** we describe how the literature references were obtained and evaluate the reported BCF values distinguishing three quality categories: very useful, useful, and not useful ones. In **Chapter IV** we report the results and separate useful BCF values into a set that can be used for correlation purposes and a set that is useful for classification purposes only. **Chapter V** addresses the issue of BCFs of mixtures since this is particularly relevant in chemicals policy. In **Chapter VI** we discuss the different parameters that (may) influence the BCF value. In **Chapter VII** the nature of the interaction between the surfactant and fish is evaluated from the current scientific literature. **Chapter VIII** evaluates possible quantitative relationships between useful BCF and reliable sorption constants of surfactants. In **Chapter IX** we discuss the implications of our assessments of surfactants in the HMCS and CHARM model the possible use of the OECD 117 protocol for assessment of BCFs. **Chapter X** provides the conclusions and recommendations of the study.

2. BCF methodologies used for surfactants

The determination of the BCF can be performed in two ways, experimentally and computationally with QSARs. However, the latter cannot be used if experimentally determined values are not available and if a theoretical concept is lacking.

2.1 Experimental methods

The BCF value is important in environmental hazard labelling and risk assessments. Consequently a standard flow-through method has been developed, the OECD 305 test. BCF-values established according to the OECD 305 are used in chemicals policy e.g. in the determination of the environmental R-phrases of a substance or in a complete risk assessment of a chemical according to the (revised) TGD 1488/94 (JRC 2003). The potential for bioaccumulation also plays an important role in the Harmonised Offshore Chemical Notification Format (HOCNF) adopted by OSPAR (1995) where a log K_{ow} cut-off value of >3 or BCF_{exp} >100[1] and considering molecular weight is used to identify potential bioaccumulating substances. In the standard method a procedure is described for characterising the BCF potential in fish under flow-through (or semi-static) test regimes. In a flow-through experiment the water concentration of the chemical remains constant, while under the semi-static test regime the water concentration varies in a tooth-saw way during the experimental time period. For kinetic modelling a constant concentration is preferred.

BCF are also measured under field conditions. The concentration of the test substance is measured in the aquatics organism and the water compartment. In general one speaks often in this case of the bioaccumulation factor (BAF) since the test substance is also susceptible to sorption to material dissolved or floating in the water compartment. Therefore the water compartment is not well defined, a pre-requisite for a proper BCF experiment. Whether BAF are useful as well can only be decided after an expert judgement. BCF in (semi)field experiments are always determined by the ratio of the concentration measured in the organisms and dissolved in the surrounding water. Therefore authors assume in general a steady state approximation. They, however, do not critically examine this condition and in this report it is indicated that in these cases the BCF is determined according to steady state conditions without sufficient evidence that steady state is attained (SS-n_{ss}).

The OECD 305 standard test consists of two phases: an uptake and a depuration phase. The concentration of the test substance in/on the fish (or specified tissue thereof) is followed through both phases of the test. The uptake phase is normally run for 28 days. A substantial part of the full time period the concentration of the substance in the fish should not show a significant variation in the concentration. In this case a steady-state situation has been reached. After reaching a steady-state situation the fish is transferred to a medium free of the test substance for the depuration phase. In general the uptake and depuration rate constants follow a simple kinetic model. From this simple model a BCF value can be derived as the ratio of the uptake and depuration rate constants, BCF_{kin}. In the steady-state situation a second BCF value can be derived by the ratio of the constant concentrations of the test substance in the fish and water, BCF_{ss}.

[1] This limit value is based on the value for environmental hazard classification according to the EU DSD (67/548/EEC). In the new classification and labeling system of the GHS that is expected to be introduced in 2007 concurrently with REACH, the log K_{ow} classification limit is set at 4 and the BCF limit value for classification purposes is set a 500 L/kg (wet weight).

The kinetic model becomes more complex if biotransformation in the test animal is included (see Annex I). The experimentally observed uptake and depuration rate constants become complex functions of all the different kinetic rate constants. However the OECD standard method does not take into account explicitly the presence of biotransformation process in the determination of the BCF according to the kinetic method and uses a simple one-compartment rate model to be used in the fitting procedure. In the kinetic method the experimental sorption curve is modelled to the one compartment kinetic scheme. Annex I shows the solution of a kinetic scheme that includes biotransformation. The resulting sorption curve should me modelled using Eq. A1. This equation is different from the simple one-compartment kinetic schem (Eq. A3). Annex I shows that the BCF determined by the kinetic method according to the (pre)equilibrium method as described in the OECD 305 leads to an overestimation compared to the BCF determined by the steady state method. This conceptual difference is the cause of the constant increase by a factor of 2.5 in BCF values of LAS by the kinetic method observed by Tolls *et al*. (1997). Therefore we conclude here that in case the BCF is determined kinetically according to the OECD 305 method the measured BCF is an overestimation of the true BCF. A true BCF for any surfactant should thus be based on the steady state method. The studied literature does not distinguish in general kinetic or steady state BCF values. If the BCF is determined by the correct kinetic scheme, the resulting BCF_{kin} is derived correctly. So far no reference has been found in the scientific literature where the correct kinetic scheme is applied.

For screening purposes as in the HOCNF scheme a kinetically determined BCF is still useful. If this value remains below the limit value set (e.g. BCF = 100 L/kg (total wet weight), then the true BCF also remains below this limit. A second important conclusion here is that the BCF of one substance depends on the type of fish or other organism used in the experimental determination. Therefore correlations based on BCF values from different organisms are not useful. One has to keep in mind that the OECD 305 has been designed for hydrophobic substances that are little susceptible to biotransformation like PCB, dioxins etc.

Many different types of organisms are being used in BCF experiments. However the OECD 305 method indicates the use of higher organisms like fish and invertebrates. Although BCF values are reported in the literature using algae and crustacean, these values have been disregarded.

2.2 Computational methods

Computational methods are easier and less time-consuming than experimental ones. In general one looks for a set of experimentally determined BCF values of a group of chemically highly similar substances (dependent values) to establish a (linear) correlation with one or more descriptors (independent values). As indicated above this is not a sufficient condition for the BCF training set since the BCF of surfactants depends at least on the biotransformation rates. Therefore a reliable data set should be based upon experimental BCF from the same organisms. The selected descriptors are often derived from the molecular structure of the substances and/or related to a theoretical concept. Since surfactants are capable of both an electrostatic and hydrophobic interaction it remains to be seen whether one of the two or both possible interaction(s) dominate(s) the bioconcentration process. We will return in more details to the established correlations in section 6 when reporting and interpreting the reported BCF correlations.

3. Method

3.1 Literature list

Literature on BCF values of surfactants before 1994 was extensively reviewed by Tolls et al. (1994). The review by Tolls and co-workers was used as a starting point for the present study and literature after 1994 was collected. Tolls and coworkers concluded that all reported BCF data were not corrected for biotransformation. Apparently only radiolabelled substances were used in all experiments and the radioactivity in the fish was measured without correction for the formation of metabolites. These reported BCF data are in general overestimations of the true BCF. However, the reported values are still useful for a (pre-)screening method. If the reported observed BCF remains below the screening limit value, then the true BCF will also remain below the limit value. Therefore their selected BCFs are useful for screening purposes and are all included in the overall BCF list of the current report. The BCFs rejected in Tolls' review were not included in the overall list. Tolls and coworkers rejected these values because in general the pertinent references did not indicate any evidence for attainment of a steady state situation. An additional CAS search completed the literature inventory. The resulting hits were compared with the references cited in the critical review of Tolls et al.

More recent reviews were also checked for additional references, more specifically the following ones:

- Bioconcentration (Saez et al 2003)
- Environmental and Health Assessment of Substances in Household Detergents and Cosmetic Detergent Products (Madsen et al 2001)

Searches in the Chemical Abstract were performed using the following keywords with in parenthesis the number of unique hits:

- Surfactant and bioconcentration (32)
- Surfactant and bioaccu OR biomagni OR bioconc (131)

All hits were checked for relevant information. One additional reference was found that was not included in the set of Tolls et al (1994). Therefore we conclude here that the search of Tolls *et al* (1994) was nearly complete.

Cited references not included in the references obtained from the CAS search were checked for extra information. It seems from the EU risk assessment of DDMAC that lab reports remain outside the CAS search. Therefore more attention was given to the grey literature. The following internet database was searched for information of BCF of surfactants:

HERA database (HERA project Risk assessments on ingredients of household products) www.heraproject.com. Very little information on BCF values was retrieved from the publicly accessible risk assessments of several household surfactants. Attempts to obtain relevant information from other sources was also not very successful.
Since the BCF is a parameter used in the classification of substances, additional relevant information might be found on a (Material) Safety Data Sheets (SDS). However, the time limit was too short too check for these SDS. In addition companies often classify the underlying reports as confidential. The reference that cited the underlying BCF of DDMAC (Juergensen 2000) could only be consulted from a microfiche.

3.2 Evaluating literature BCF values

The quality of the BCF values reported was assessed similarly as in the Aquapol project (Krop et al, 1995). In the Aquapol project a list of analytical, methodological and statistical criteria was established for several environmentally relevant endpoints among which the BCF. Each criterion was assigned a weight factor determined by an expert group. The complete set of criteria is given in Annex 2. Each criterion is then assessed by a *yes* if the criterion was fulfilled, *no* if it was not fulfilled, *unknown* if it should have been fulfilled according to the experimental design but the pertinent reference was not clear enough and *not applicable* if the criteria is not relevant for the method used in the reference. *No* and *unknown* are counted as a negative outcome, while *not applicable* is not accounted for in the overall result. The overall result is then given in a score between 0 and 1. A high score indicates that most criteria are met. The completeness score is not stated in the overall resulting BCF database but it estimates to what extent the cited reference corresponds to the concepts of the database. The three different sets of criteria lead to three scores that are included in the BCF database for reported BCF values after 1994.

The overall quality and usefulness of the cited BCF values are determined as follows. The following criteria were applied where each criteria was assigned an equal weight factor of 1.

1) BCF values determined by attainment of steady state (see section 2.1)

2) The surfactant in the water and organism should have been analyzed directly

3) The statistical assessment score should be higher than 0.50. This guarantees that sufficient data points are determined that allows a proper statistical evaluation of the steady state and the BCF value.

Therefore each BCF value may obtain an overall quality between 0 and 3. We consider BCF values with an overall quality of *3 as very useful*, of *2 as useful* and *<2 as not useful*. Each reported BCF value in Annex 3 includes the overall score.

4. Results

4.1 Overall result

Annex 3 reports all BCF values from literature since 1994, including their quality. The overall number of BCF values entered in the final database is divided over the different types of surfactants as follows:

Anionic	140
Cationic	82
Non-ionic	35
Amphoteric	0
Total	**257 reported BCF values**

The *anionic* surfactants are divided into the following groups:

AS (C12)	4
Perfluorsurfactanten	8
Laurate	1
LAS (C11 – C13 and 2 – 5 position)	127

It is clear that LAS contributes to 91% of the reported values.

The *cationic* surfactants are divided as follows:

Quarternaire Ammonium compounds	12
Long chain alkylnitriles	24
Long chain trialkylamines	46

However 85% (all long chain alkylnitriles and trialkylamine) of the reported values are from one reference only (Valis et al 1989)

The *nonionic* surfactants belong to the group of the C12 – C14 alcohol ethoxylates and to the group of nonylphenol ethoxylates. A total of 35 BCF values were reported of which 21 referred to AEO and 12 to short chained NPEO (EO ≤3). Reported NPEO values in reviews were all before 1994 and were discussed in the review of Tolls et al (1994). If included in his list of useful values they are included in the overall BCF list (Annex 3). One single ref after 2000 was found where BCF of NPEO were determined. Fortunately the experiment was conducted in marine waters.

No values were found for *amphoteric* surfactants.

4.2 Useful BCF values

BCF values can be used in two ways: a) as a value that is used in an environmental risk assessment and b) as a screening value. In a risk assessment a reliable BCF value is normally used. These BCF are derived from experiments according to the OECD 305 where the substance has been analysed directly both in the species (fish) and in the water. In this report we indicate these BCF as (very) useful. However literature also reports BCFs that are derived in other ways. In most ways these values are upper or lower approximations of the useful BCF. These values are, however, not useful for a risk assessment but can still be useful for screening purposes. These BCF give may give valuable information for classification purposes. The new Globally Harmonised System (GHS) of classification and labelling of

substances and mixtures, that will most likely be implemented in the EU concurrently with the new chemicals policy REACH, specifically describes how to use these type of BCF (GHS 2005, Annex 9, A9.5.2.3.9) in this classification system. This specific information can be found in Annex 5 of this report. The reported BCF of the anionic surfactants C12-Alkylsulphate is an example where the indirect analytical method leads already to such a low BCF that the BCF determined from a direct analysis of the surfactant in both compartments (fish and water) will unlikely result in a value that would exceed the limit of 100 L/kg (or 500 L/kg in the GHS system).

4.2.1 (very) useful BCFs of surfactants

Annex 3 shows all BCF values retrieved from the references. The Annex 3 list is separated into their respected surfactant classes. Table 4.1 gives the set of 41 (very) useful BCF values according to the applied criteria (section 3.2). This set will be used as a starting set in the correlations of the BCF with the sediment water partition constants. Nearly all BCF are determined in river water systems. The ones reported in marine water are indicated in Table 4.2.

Substance name	type of surfactant	Analytical method	Data analysis	Stat	Overall result	Reported true BCF value (L/kg) (wet weight)	English name of specie	Ref
Laurate (sodium)	an	Direct (LSC, GC-FID)	SS	0.45	2	**255**	Zebra fish	Egmond (1999)
C12-EO8	non	Direct (HPLC-Flu det)	SS	0,31	2	**12.7**	fathead minnow	Tolls, 2000 B
C13-EO4	non	Direct (HPLC-Flu det)	SS	0,31	2	**232.5**	fathead minnow	Tolls, 2000 B
C13-EO8	non	Direct (HPLC-Flu det)	SS	0,31	2	**40**	fathead minnow	Average (5)
C14-EO4	non	Direct (HPLC-Flu det)	SS	0,31	2	**237.0**	fathead minnow	Tolls, 2000 B
C14-EO8	non	Direct (HPLC-Flu det)	SS	0,31	2	**96**	fathead minnow	Average (2)
C14-EO11	non	Direct (HPLC-Flu det)	SS	0,31	2	**15.8**	fathead minnow	Tolls, 2000 B
C14-EO14	non	Direct (HPLC-Flu det)	SS	0,31	2	**<5**	fathead minnow	Tolls, 2000 B
C16-EO8	non	Direct (HPLC-Flu det)	SS	0,31	2	**387.5**	fathead minnow	Tolls, 2000 B
C10-2-LAS	an	Direct (RP-HPLC-fluor)	SS	0,3	2	**1.4**	Rainbow trout	Tolls 2000C
C10-2-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**6.0**	fathead minnow	Tolls, 1997
C10-in-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**3.0**	fathead minnow	Tolls, 1997
C11-2-LAS	an	Direct (RP-HPLC-fluor)	SS	0,3	2	**6**	Rainbow trout	Tolls 2000C
C11-2-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**31.9**	fathead minnow	Tolls, 1997
C11-5-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**6.1**	fathead minnow	Tolls, 1997
C11-5-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**9.8**	fathead minnow	Tolls, 1997
C11-in-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**9.1**	fathead minnow	Tolls, 1997
C12-2-LAS	an	Direct (RP-HPLC-fluor)	SS	0.30	2	**82**	Rainbow trout	Tolls 2000C)
C12-2-LAS	an					**153**		Tolls, 2000C
C12-2-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**99.1**	fathead minnow	Tolls, 1997
C12-2-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**168.4**	fathead minnow	Tolls, 1997
C12-2-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**211.5**	fathead minnow	Tolls, 1997
C12-2-LAS	an	Direct (HPLC-fluor)	SS	0.77	3	**222**	fathead minnow	Tolls 2000A

C12-2-LAS	an	Direct (HPLC-fluor)	SS	0.32	2	**138**	fathead minnow	Tolls 2000A
14C-C12-2-LAS	an	Direct (LSC/TLC)	SS	0.45	2	**39**	Midge	Hwang 2003
C12-2-LAS	an	Overall reported value				**153**		Tolls 2000
C12-3-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**42.1**	fathead minnow	Tolls, 1997
C12-5-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**10.0**	fathead minnow	Tolls, 1997
C12-6-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**31.9**	fathead minnow	Tolls, 1997
C12-in-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**29.9**	fathead minnow	Tolls, 1997
C13-2-LAS	an	Direct (RP-HPLC-fluor)	SS	0.30	2	**372**	Rainbow trout	Tolls 2000C
C13-2-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**987.2**	fathead minnow	Tolls, 1997
C13-5-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**34.0**	fathead minnow	Tolls, 1997
C13-in-LAS	an	Direct (HPLC-fluor)	SS	0.58	3	**112.5**	fathead minnow	Tolls, 1997
Pfoctanoic acid	an	Direct (LC-MS-MS)	kinetic	0.45	2	**4.0**	Rainbow trout	Martin, 2003 A
Pfdecanoic acid	an	Direct (LC-MS-MS)	kinetic	0.50	3	**450**	Rainbow trout	Martin, 2003 A
Pfundecanoic acid	an	Direct (LC-MS-MS)	kinetic	0.70	3	**2700**	Rainbow trout	Martin, 2003 A
Pfdodecanoic acid	an	Direct (LC-MS-MS)	kinetic	0.70	3	**18000**	Rainbow trout	Martin, 2003 A
Pftetradecanoic acid	an	Direct (LC-MS-MS)	kinetic	0.70	2	**23000**	Rainbow trout	Martin, 2003 A
Pfoctane sulfonate	an	Direct (LC-MS-MS)	kinetic	0.50	3	**1100**	Rainbow trout	Martin, 2003 A
Pfhexanesulfonate	an	Direct (LC-MS-MS)	kinetic	0.70	3	**9.6**	Rainbow trout	Martin, 2003 A

Table 4.1 Useful BCF values in river water organisms according to the applied criteria.

Substance name	**type of surfactant**	**Analytical method**	**Data analysis**	**Stat l**	**Overal result**	**Reported BCF value (L/kg)**	***Latin name of specie***	**English name of specie**	**Exp concentration**	**Ref**
C11-LAS (74% -3-)	an	Direct HPLC-fluor	SS	0.14	2	**40**	*Ruditapes semidecussatus*	Clam	60 µg/L	Saez 2002
C11-LAS (74% -3-)	an	Direct HPLC-fluor	SS	0.14	2	**36**	*Ruditapes semidecussatus*	Clam	190 µg/L	Saez 2002
C11-LAS (74% -3-)	an	Direct HPLC-fluor	SS	0.14	2	**37**	*Ruditapes semidecussatus*	Clam	350 µg/L	Saez 2002
C12-2-LAS	an	Direct HPLC-fluor	SS	0.14	2	**1120**	*Ruditapes semidecussatus*	Clam	30 µg/L	Saez 2002
C12-2-LAS	an	Direct HPLC-fluor	SS	0.14	2	**370**	*Ruditapes semidecussatus*	Clam	100 µg/L	Saez 2002
C12-2-LAS	an	Direct HPLC-fluor	SS	0.14	2	**380**	*Ruditapes semidecussatus*	Clam	140 µg/L	Saez 2002
NPEO2.8	non	Direct HPLC-fluor	SS	0.14	2	**4460**	*Ruditapes semidecussatus*	Clam	3 µg/L	Saez 2002
NPEO2.8	non	Direct HPLC-fluor	SS	0.14	2	**3700**	*Ruditapes semidecussatus*	Clam	4 µg/L	Saez 2002
NPEO2.8	non	Direct HPLC-fluor	SS	0.14	2	**3960**	*Ruditapes semidecussatus*	Clam	8 µg/L	Saez 2002

Table 4.2 Useful BCF values in marine organisms according to the applied criteria.

4.2.2 Surfactants below or above the BCF limit value of 100 L/kg or 500 L/kg

a) BCF < 100 L/kg

We add here a second list of surfactants of which the experimental values indicate that the true BCF will be below or above the desired limit value. For screening purposes according to

the HOCNF a BCF limit value of 100 L/kg (wet weight) is agreed upon. This is similar as for classification purposes according to the DSD. However, on introducing the GHS system in 2007 in Europe, the classification cut-off value for bioaccumulation will raise to 500 L/kg (wet weight). Therefore we will consider two cut-off values, 100 L/kg and 500 L/kg respectively. Surfactants (not included in Table 4.1) with a reliable cut-off BCF < 100 L/kg are:

For the following *anionic* surfactants:

- Alkylsulphates with alkyl chain length < 12

For the following *cationic* surfactants:

- DialkylDimethylAmine (DDAC) surfactant with both alkylchains >10 C-atoms
- Alkyltrimethylamine surfactants (TMAC) with alkyl chain length <12 C-atoms

No BCF values are found that are below 500 L/kg but above 100 L/kg. Therefore raising the bioconcentration limit to 500 L/kg does not include new surfactants in this group.

b) BCF > 100 L/kg

- Laurate-ion (BCF = 255 L/kg)
- Perfluoralkanoic acids with C>8
- Perfluoralkane sulfonates with C>6
- TMAC (BCF > 1000) with the addition that this is an indirect value where not corrected for biotransformation.

c) BCF > 500 L/kg

- Perfluoralkanoic acids with C>10
- Perfluoralkane sulfonates with C>6

4.3 Additional information on reported BCF values

4.3.1 Anionic surfactants

LAS

Tolls et al (2000C) report to use an overall fresh water BCF value of C12-2-LAS of **153 L/kg** based on 79 observations. One has to realize that this value is based on fresh water fish and these fish do metabolize LAS. However, a directly determined BCF value of C12-2-LAS in the midge (Hwang 2003) that does not metabolize LAS is reported to be 240 L/kg. This value decreases six-fold in case feeding was introduced in the experimental set-up. Since commercial LAS consist of a mixture of C10 – C14 alkylbenzenesulphonates, it cannot be concluded from Table 4.1 that the corresponding BCF value of the fresh water BCF of a LAS mixture is below 100. Therefore we conclude here that the BCF of commercial LAS need to be calculated properly using the reported BCF values of Table 4.1.

Fluorinated surfactants

Perfluorinated surfactants have gained quite some interest lately since they are used extensively in a number of industrial and commercial applications like lubricants, paints, polishes, food-packaging and fire-fighting foams. Concerns regarding their environmental persistence and the bioaccumulation potential prompted suppliers to look for alternatives. The fact that they the carbon backbone is nearly completely fluorinated indicates that their potential for oxidation is extremely reduced. This is the main reason that these surfactants are

not metabolised in living organisms similar as highly chlorinated and brominated carbon compounds. The carbon – fluor bond is the strongest of the covalent carbon – halogen bonds therefore making it kinetically very unfavourable to break.
Martin (2003A) reported some useful values for a set of fluorinated surfactants in lab water. Owing to the fact that fluorinated surfactants are not metabolised, the normal kinetic scheme as described in the OECD 305 is appropriate to derive the correct BCF value. The time to reach steady state was shown to be quite long as is expected for these strongly hydrophobic compounds.
Moody et al (2002) estimated a BCF range for perfluorooctanesulfonate (PFOS), based on field measurements of surface water and fish samples in the order of 6300 to 125000 L/kg. However, they indicated that the high concentration of PFOS in the fish liver tissue may have been caused by the metabolization of other PFOS-derivates and therefore bias these field values. Thus these estimated values should be treated with caution although it is expected that the BCF is high. However the EU agreed recently to restrict the use of PFOS severely (IP/06/1479 Date: 25/10/2006). Therefore limited attention is paid to fluorinated surfactants.

4.3.2 Cationic surfactants

Dioctadecyl DimethAmine chloride

The EU Risk assessment (ECB 2002) of Dimethyl Dioctadecyl Ammonimum Chloride (DODMAC) concluded that based on test results with laboratory water, a bioaccumulation is indicated, but it is assumed that it is low under environmental conditions. A (fresh water) **BCF** of **13 l/kg** is used in the risk assessment (related to PEC_{bulk}), assuming fish to be representative for all aquatic organisms. This value is reported in the review of Tolls et all (1994) but based on an indirect analysis of the surfactant. It should be pointed out, that for the diversity of organisms and environmental conditions the bioaccumulation potential (bioconcentration and biomagnification) is not known. A relatively simple microcosm study might clarify these uncertainties. Owing to the high molecular mass of DODMAC (~ 600 g/mol) that approaches the pass membrane limit of such molecules (~800 g/mol) it cannot be concluded whether DDACs with alkyl chain lengths lower than 18 C-atoms will have lower BCF. It must be stated here that a reported BCF of 81 L/kg of DDAC (with carbon chains of 10 C-atoms only) as a reference is stated in the literature (Juergensen 2000).

Monoalkyl TrimethAmine chloride (TMAC)

All but one reported BCF values of TMAC (Monoalkyl trimethyl ammonium compounds) with an alkyl chain length of 12 C-atoms or less, are below 100 L/kg (in fresh water) apart from one reference (BCF = 104 L/kg). All these values have been determined indirectly. Thus we conclude here that the BCF of TMAC with an alkyl chain length of 12 C-atoms or less pass the BCF limit (HMCS) of 100 L/kg.
The BCF of the TMAC with an alkyl chain length of 16 – 18 C-atoms, however, may exceed the limit of 100 L/kg or even 500 L/kg. The BCF of TMAC seems to be a factor of 10 – 30 higher than its corresponding dialkyl compound [C(16/18)2-DDAC]. It is remarkable that the monooctadecyl compund may possess a higher BCF than the dioctadecyl one. A reason could be the rather high molecular mass of the dioctadecyl compound of nearly 600 g/mol. It is well known that molecules with a high molecular mass do not pass the biological membranes or pass the membrane very slowly. Thus if the BCF is influenced by the molecular masses approaching the value of 800 g/mol, the BCF of [C(16/18)2-DDAC] is expected to be lower than the one of TMAC. Valis et al (1989) also suggest that this is the reason why the higher alkylated LAN (Long-chain alkylnitriles) and TAM (long-chain trialkylamines) are present in very low concentrations or are non detectable in a number of sea organisms.

The quarternary ammonium compounds are positively charged. Owing to their charge it is expected that their BCF are (much) lower than the corresponding neutral amine compounds. Estimated BCF in seawater indicate this behaviour (Valis et al 1989). Compare e.g. CH3N(C16H33)(C18H37) with reported BCF varying between 1500 and 7000 L/kg in sea water depending on the type of organism and the overall BCF in fresh water of 13 L/kg of the ammonium compound selected by the EU. However the quality of the BCF values of Valis is low. In additions the influence of the salt ions in water on the BCF endpoint is unknown.

4.3.3 Non-ionic surfactants

The average BCF value of the nonionic surfactant C14EO7 in fresh water organisms is estimated by the authors to be around 730 L/kg (Tolls1994). However, correcting for metabolism in the analysis lowers the BCF of C14EO8 to an estimated 110 (Tolls 2000A).

Tolls et al (2000B) determined an overall fresh water BCF of C13EO8 of 39.6 L/kg from their experiments. Owing to the relatively small standard error in the 85 values of 1.6 L/kg they concluded that the average fresh water BCF was determined with considerable precision. We suggest that his dataset is useful in estimating overall fresh water BCF at least for classification purposes. The set was used to estimate the BCF of an AEO mixture determined by Evans (1994). The overall result shows a BCF of 142 L/kg, indicating that AEO mixtures may not pass the limit value of 100 L/kg in the HMCS scheme.

It is not allowed anymore to use of alkylphenol polyethoxylates (APEO) in the offshore industry. Therefore BCF of APEO have not been actively searched for but the ones that are reported in reviews and that are determined concurrently with other surfactants are assessed and included in the overall list (Annex III) in order to complete the BCF table. These values are still useful for correlation purposes.

4.4 Consequences of using the reported BCF values

The log K_{ow} and, in case this endpoint is not available or cannot be defined properly its BCF, is used for establishing the environmental hazard classification of a substance. However, the way CESIO (Cefic 2003) uses the available information on surfactants to avoid such a classification is at least questionable owing to the following arguments:

1. According to their report there is an imbalance in established BCF values (see section 4.1). Based on this imbalance and the observed biotransformation processes in these cases, CESIO recommends not touse a precautionary measure for surfactants (in general). However such a conclusion is by far too optimistic and is easily carried forwards as a kind of proof that surfactants do not pose an environmental risk. Reported studies on alkylphenol ethoxylates and fluorinated surfactants already show that such a conclusion cannot be made in general.
2. CESIO also conclude that the BCF of surfactants cannot be measured. This conclusion is incorrect as is shown in the scientific literature. Experimental problems may be expected in the analytical determination of the specific surfactant (class) but not in the BCF method itself.
3. CESIO also uses the position of the OECD Expert Group stating that there is no need to test the bioaccumulation potential of chemicals if they are readily biodegradable. While this is a position of an expert group, it is still in contradiction to the EU Dangerous Substance Directive (DSD 67/548/EEC). That the BCF limit value is due to change is expected when the Globally Harmonized System (GHS) will be introduced. In the GHS (GHS 2005) the limit value of the BCF for classification due chronic aquatic toxicity will be set at BCF >500 L/kg (wet weight) and a potential for bioaccumulation based on a log

K_{ow} >4. However, this limit still needs to be considered irrespectively of the fact whether the surfactant is rapidly biodegradable (GHS terminology). Thus if the surfactant shows to possess an acute aquatic toxicity between 1 – 10 mg/L and is rapidly biodegradable but shows an experimental BCF > 500 L/kg (wet weight), the surfactant still needs to be classified as hazardous for the aquatic environment category chronic 2 despite its rapid biodegradability. Since the OSPAR pre-screening limit values are in line with the current EU regulation (OSPAR 2000/4), they will most likely be revised as soon as the GHS system enters into force in Europe.

In addition since the BCF is a critical parameter in the assignment of the environmentally hazard phrases (R50 – R53) it might be expected that in future experiments, selected organisms with a high metabolizing rate constant will likely to dominate the experiments rather than the relevant organisms in the water compartment.

We conclude from this chapter that:

1. Nearly all BCF values are determined in fresh water systems. There is substantial lack of useful BCF determined in marine water organisms.
2. A total set of 257 BCF values could be created from the literature. 54% refer to anionic, 32% to cationic and 10% to nonionic values. From the 54% anionic 91% referred to LAS and from the 32% cationic 85% were retrieved from 1 reference of low quality. No BCF were reported for amphoteric surfactants.
3. From a total set of 257 BCF values of surfactants only 56 (20%) are of sufficient quality: 8 belonging to the AEO, 3 to one short chain NPEO, 7 to the fluorinated group, 1 to a fatty acid and 24 to LAS. Some LAS components have different useful BCFs. We have not combined these values to an overall one.
4. The fathead minnow is the mostly used fish specie for BCF experiments. Since most surfactants are metabolized in the organism, the choice of the organism is critical in the outcome of the BCF value.
5. The reported BCF values are separated into a group of useful values according to a set of criteria and in a group of values that can be used for classification purposes since the measured BCF values are considered to be an over- or underestimation of the unknown true BCF.
6. The reported BCF values are not used sufficiently for EU classification purposes.

5. BCF of mixtures of surfactants with a single EINECS/ELINCS or CAS number

Surfactants are often considered to be one substance in chemicals policy; they have a single EINECS or CAS number. Chemically seen they are composed of a mixture of different surfactant molecules. Thus it is important to develop a method how to treat such a chemical mixture. In general one starts in using the independent interaction model, i.e. the total effect is caused by simple addition of the relative contribution of the individual interaction. Therefore it is important to know whether a single interaction picture for surfactants can be applied. Tolls et al have addressed this problem for LAS and AEO. We briefly report the results.
Tolls et al. (1997, 2000B) defined the BCF of the LAS and AEO mixture as follows, Eq 5.1:

$$BCF_{mix} = \frac{\sum C_{f,i}}{\sum C_{w,i}} \tag{5.1}$$

where $C_{f,i}$ is the concentration of the LAS component *i* in the organism and $C_{w,i}$ in water respectively. They showed that the BCF value of a mixture can best be represented by the addition of the fraction present in the water phase, $\phi_{i,w}$, and the relative BCF-value of the specific component, Eq. 5.2. This supports an independent interaction model.

$$BCF_{mix} = \frac{\sum C_{f,i}}{\sum C_{w,i}} = \sum \left(\phi_{i,w} * BCF_{i,rel} \right) \tag{5.2}$$

Although not completely correct in this case since the BCF value is derived from a steady state system and not from an equilibrium one, Eq. 5.2 is in agreement with the thermodynamic approach of obtaining an equilibrium value of a mixture from its components. This is important since the equilibrium constant, K, is related to the free enthalpy change of the process (ΔG^0) by $\Delta G^0 = - RT \ln K$.

On using Eq 5.2 authors estimated in this way an overall BCF value of an effluent mixture characterised by Evans (1994) to be 142 L/kg based on the individual BCF values of the AEO. This mixture, if characterised as a single substance in chemicals policy has a BCF above the limit value above the 100 L/kg.

CESIO (CEFIC 2003) indicates that there is no established procedure available to calculate a BCF for a complex substance like a surfactant composed of a mixture of homologues. They conclude that a BCF cannot be given. However, while it may not be an *established* procedure the EU Dangerous Substance Directive (67/548/EEC) insists on using all available information in the classification and labelling process and not only established procedures. Scientific literature indicates that a procedure is known, as outlined above, and should therefore be used in the classification and labelling purposes.

We conclude that:

- the BCF of a surfactant composed of a mixture of homologues can be determined by summing the contributions of the fractions of the individual components and its corresponding BCF.

6. Parameters that influence the BCF value

6.1 Steady state vs kinetic

We have reported already that the BCF value can be different if derived from the steady state values or from the uptake and depuration rate constants. The kinetically determined BCF values from LAS have been reported to be consistently higher by a factor of 2.5 (Tolls et al 1997). We have suggested that the steady state values are more appropriate then the kinetic ones unless the correct kinetic scheme is used.

6.2 Feeding behaviour

A second source of uncertainty and variation in BCF values is caused by the differences in feeding regimes applied. Newsome (1995) indicates that elimination of LAS and AEO was significantly slower in unfed goldfish than in fed ones due to decreased bile production and secretion into the gut, which is the primary route of excretion for the surfactants. For both type of surfactants biotransformation seems to occur via ω-oxidation followed by β-oxidation. The oxidation steps seem therefore to be rather unspecific for both LAS and AEO. Thus feeding seems to lower the BCF values for LAS and AEO.

Hwang et al (2003) distinguished two estimates of BCF values of LAS, one from the toxicokinetics exposures without feeding and one estimated at the end of the chronic test conducted with feeding. The BCF values differed by a factor of about six (240 L/kg vs 40 L/kg) with the water-only exposures generating the higher estimate. The authors suggested that the eliminated LAS are adsorbed onto the food, leading to an increase in the elimination rate and reduction of the BCF.

Martin (2003 B) reported that the dietary accumulation of perfluorinated surfactants does not only depend on the hydrophobic character but also on the type of head group. However this is incorrectly assigned to the accumulation process since sorption of the surfactant to the food is not linear. Therefore the overall accumulation factor is a combination of bioaccumulation and non-linear sorption process. In addition the Dietary accumulation factor (BAF) for the set of perfluorinated surfactants in juvenile rainbow trout did not exceed 1.0 (kg/kg) indicating that this uptake route is most likely not very significant.

6.3 In situ BCF values

Field measurements are expected to result in lower BCF values than lab values according to the OECD 305 method. One of the factors that lowers the BCF is the presence of feeding the *in situ* experiments. However results of LAS reported in the literature (Tolls EST 2003) estimates field BAF-BCF values above lab BCF values in a caged fish experiments. Although no explanation could be found it was suggested by the authors that these higher values are experimental artefacts in that LAS sorbed to suspended solids in the gills of the fish may have caused the increase of the amount of LAS analysed in the fish. These finding are in contrast with findings reported by Sáez (2002) in marine waters in the Cadiz bay in Spain. In this case the in situ values were between a factor of 10 – 20 lower for LAS, of 3 – 7 for $NPEO_{1-10}$ and of 20 for Nonylphenol than her measured lab values.

6.4 Metabolism of the test substance

A third possible source of variation is the difference in metabolic rate constants between different organisms. This makes the BCF to depend on the type of fish or other organism used. In additional experiments Tolls et al (2000C) showed that the BCF values of LAS derived from steady state systems with rainbow trout are in general lower than with the fathead minnow. They assigned this difference to a difference in biotransformation rates,

being higher in case of the rainbow trout. This is in agreement with the simple kinetic model as described in Annex 1 where biotransformation rate constants are included in the scheme as k_2. Literature reports that three organisms cannot metabolise LAS; the midge, the fish Hyalella and Channel catfish (Hwang 2003). For other surfactants it is unknown yet which fish cannot metabolise any of the other surfactants apart from fluorinated surfactants. Fully halogenated surfactants (by fluorine, chlorine and/or bromine) are fully oxidised by another oxidator than oxygen and are therefore very little susceptible to oxidation processes.

6.5 Exposure concentration

Versteeg et al (2003) suggested, contrary to Tolls et al (2000C) that the (average) BCF values depend on the exposure concentrations, a possible fourth source of variation. The exposure concentrations in the experiments performed by Versteeg et al. (2003) vary between 4 and 90 µM and are near the LC50 values of the fathead minnow, Hyalella and Channel catfish. In case of the *Corbicula* and *Elimia* such a comparison could not be made since the reported LC50 values were indicated only as > 3.0 mg/L. The reported exposure concentrations for LAS of Versteeg et al (2003) are higher by almost a factor of 10 – 100 than those used in the experiments of Tolls et al (2000C). We cannot conclude here that the reported BCF variation is statistically significant since the necessary statistical parameters are lacking.

The BCF experiment of LAS performed by Tolls et al (1997) was divided into 4 batches with different LAS components. However, in each batch one LAS component was similar, the C12-2-LAS to account for any variation between the batches. The measured BCF values for C12-2 were significantly different from each other. The authors could not explain this variation. Madsen (2001) suggested that these differences were caused by a variation in the concentrations. Tolls reported the sum of the concentrations of the individual test compounds ($\Sigma C_{w,i}$) in the exposure water during steady state ranges between 2.7 and 4.1 µM. The overall concentration is therefore quite constant but the fraction C12-2 varies between 0.005 and 0.165 and do corresponds with a decrease of BCF values. Sáez report some decrease in BCF values on increasing surfactant concentrations but the variation in surfactant concentration was lower than a factor of 10 and the reported BCF values were not accompanied by a statistical analysis. We suggest here that the reported decrease may have been caused by an increase in metabolic activity due to the higher exposure concentrations that may even occur at low concentrations. However, there is at this moment insufficient evidence whether the exposure concentration in general does or does not influence the BCF value. Therefore the conclusion that the overall BCF value for C12-2-LAS of 153 L/kg is independent of the exposure concentration as reported by Tolls et al (2000C) should be treated with caution.

6.6 Molecular mass

A fifth source of variation is the influence of the molecular mass of the compound. It is generally accepted that above a specific molecular mass no toxic effect is observed since the test compound cannot pass a biological membrane anymore. The OSPAR decision scheme does not consider a molecular mass value. In one of the earlier versions of REACH a mass limit of 800 g/mol was proposed that has been used in the Ecolable criteria for lubricants (http://ec.europa.eu/environment/ecolabel/product/pg_lubricants_en.htm) downloaded January 2006). In the official proposal of REACH the limit value of 800 g/mol disappeared. This is, however, in line with recent publications on the uptake of brominated flame retardants with a molecular mass higher than 800 like Decabromodiphenylether (BDE 209) in e.g. juvenile Rainbow trout and Common carp (Voorspoels 2003, de Wit 2005, Stapleton 2006). BDE 209 has a molecular mass of 959 g/mol. Therefore the limit of 600 g/mol indicated in the CHARM manual version 1.4 may also be reconsidered.

6.7 Ionic strength

The influence of the ionic strength on the BCF is difficult to determine at this moment. Literature reveals few BCF determined in seawater and marine fish species. Tolls et al (2000) reported an increase of uptake rate constants due to an increase of ionic strength of the solution. However the increase was more pronounced for the more hydrophilic LAS components and was not significantly different anymore when the estimated log K_{ow} (Roberts 1989) reached a value of 4.0 or more. The difference was only pronounced when the ionic strength increased from 0.46 mM (Me^{2+}) to 1.21 mM (Me^{2+}). No significant variation was found upon further increasing the ionic strength to 3.63 mM (Me^{2+}). Although authors tried to explain the variation with the Guoy-Chapman theory of the electrical double layer (Gennis 1989) they concluded that this might only partially explain the variation. However authors did not investigate the role of activity coefficient, γ, of LAS in these experiments. The difference in activity coefficient between river water and seawater due to the presence of ions is often the cause of the difference in results of similar equilibrium endpoints e.g. solubility established in both types of water. The measured BCF values in marine water by Sáez (2002) for C11- and C12-LAS, and $NPEO_{2.8}$ show higher values than equivalent ones measured in river water. However a bivalve, i.e. an invertebrate organism is used instead of a fish species. Bivalves in general have lower transformation capacities than vertebrates [ref]. Since the influence of the biotransformation rate is not included in the overall value no conclusion can be drawn as to the influence of ions present in the water.

The difference hinges on the fundaments of the bioconcentration endpoint. The question to be answered in this case is whether the BCF is a thermodynamic endpoint and therefore depends on the activity of the component in each compartment or a kinetic endpoint, which depends on the concentration of the substance. Therefore we conclude here that a simple algorithm to establish a BCF in seawater from river water cannot be made yet. This is also in line with the fact that BCF values in seawater organisms are extremely scarce.

6.8 Dissolved organic carbon

Versteeg et al (1992) reported a number of indirectly determined BCF values that depend on the presence or absence of dissolved organic carbon (DOC). The lower BCF values are found if DOC is present in the solution. The influence of DOC present in the system has not been investigated further. Therefore we do not know at this moment whether DOC influences the BCF for surfactants. It is likely however, that DOC influences the availability of surfactants for bioconcentration. However, the concentration of DOC in marine waters is varying somewhat between the seasons but is on an average quite low, 0.6 mg C/L (Laane 1982). Therefore it is not expected that BCF values of marine organisms will depend on DOC concentration.

6.9 Other unknown sources of variability

Tolls et al (ETC 2000) performed 4 similar experiments using different groups of AEO with C13EO8 in all 4 experiments. The BCF values of this substance in these four different experiments varied between 26 and 55 L/kg. Although they suggested that the variation should be caused by an unknown mechanism, the statistical analysis showed that these values are not significantly different from each other within the 95% confidence interval and therefore an overall value of 40 L/kg is justified.

We conclude from this chapter that:

1. Biotransformation and feeding regime reduces the BCF at least for LAS and AEO. Both reducing effects are related to each other but the exact relation is unknown.

2. Biotransformation rates of a surfactant differ between each species. Literature also reported organisms, including two fish that cannot metabolise LAS. Therefore the BCF endpoint cannot be regarded as a substance endpoint only.
3. There is some indication that the BCF of surfactants depend on the exposure concentration. It seems that the BCF increases on decreasing the exposure concentration.
4. BCF generally increases with increasing mol mass, but at high molecular masses this relationship no longer holds. Different cut-off values have been proposed in the past but were always reconsidered owing to new scientific evidence. Recently, for example, BDE 209 (molecular mass of 959 g/mol) has been shown to bioaccumulate in several fish species.
5. A simple algorithm to estimate the BCF in seawater from a BCF in river water cannot be given yet. This is caused by lack of (useful) BCF data of seawater organisms and river water organisms.
6. The influence of DOC on the BCF of surfactants is unknown at this moment but is expected to play only a marginal role in the marine environment.

7. Nature of the interaction between surfactant and fish

7.1 Introduction

The nature of an interaction process is often deduced from correlations between measured values of the endpoint (the dependent variable) e.g. BCF, LC50, k_1, and relevant descriptors, e.g., the $K_{ow,}$ N_{CH2}, etcetera (the independent variable). These correlations are so-called Quantitative Structure-Activitity Relationships (QSAR). For the BCF, log BCF – log K_{ow} correlations are often cited in the literature. As indicated before, by taking the logarithm of the endpoint, the value is transferred to corresponding free enthalpy change of the process. Therefore a log BCF – log K_{ow} can be compared to a correlation of the free enthalpy change of the BCF process and the free enthalpy change of the K_{ow} process and such correlations are simple applications of thermodynamics. The nature of the independent variable gives then information on the nature of the dependent variable. In this way one can obtain information on the type of interaction that governs the unknown process. The quality and the success of such relationships depend on statistical parameters of which the standard error of the regression (s.e.r), the (adjusted) regression coefficient (R_{adj} or R^2_{adj}) are the most cited ones. If the established QSAR is used to estimate unknown values, then the QSAR must be validated. For classification purposes this validation process is important since estimation of unknown values is almost invariably the purpose of the QSAR. The quality of reported BCF correlations has beens analyzed in the present study in the same way as described previously (Krop et al 2006). The set of criteria used for this evaluation can be found in Annex 4.

7.2 Reported BCF correlations

First of all it is rather surprising to see that correlations of BCF of surfactants with e.g. the estimated log K_{ow}-values actually exist. The K_{ow} is an equilibrium property while the BCF is a steady state and therefore a kinetic endpoint. As mentioned earlier the BCF depends on the biotransformation rate constants of the test compounds. Testing a class of surfactants like LAS or AEO, one should expect therefore that the biotransformation rate is in a first approximation different for each homologue and therefore any existing correlation with an equilibrium parameter is expected to disappear The fact that BCF values of LAS and AEO do exhibit positive correlations with equilibrium descriptors like log K_{ow} suggests that the biotranformation rates of LAS and AEO in the different organism are independent of the homologue. For both type of surfactants biotransformation seems to occur via ω-oxidation followed by β-oxidation. The oxidation steps seem therefore to be rather unspecific for both the type of LAS and AEO (Newscome 1995 and this supports the existence of BCF-K_{ow} correlations).

Tolls et al (1997) showed that relative BCF values of different LAS components (relative to the 2- or 5-isomers) correlate strongly with the estimated octanol-water partition coefficient, log K_{ow}, of the individual LAS components. They used the estimation method of Roberts (1989). We report here briefly our correlation attempt of the best available log BCF values from Table 4.1 for different LAS component and the log K_{ow} established by Roberts (1991). We do not change the BCF values into relative BCF ones similar to what Tolls et al (1997) have done. Fig 7.1 presents the regression equation and R^2 of the correlation.

Fig 7.1. shows a reasonable correlation (R^2 = 0.84) of the useful log BCF values of the different LAS components from Table 4.1 and Table 4.2. However a substantial scatter remains, most likely caused by the other factors that influence the BCF value like the biotransformation rates in the different organisms e.g the BCF of 1120 L/kg for the clam in seawater from Sáez (2002). The log K_{ow} limit in the HMCS for the potential bioaccumulation

is 3. This criterion would exclude a substantial number of LAS components. The BCF limit value for potential bioaccumulation is 100 L/kg, which according to Fig 7.1 corresponds to a log K_{ow}–value of approximately 3.6. Therefore less LAS components would be excluded. The fact that the BCF value is lower than expected is caused by the presence of biotransformation of the LAS in the type of fish used. The limit values used for classifications are based on correlations from strongly hydrophobic substances without or with a very limited biotransformation.

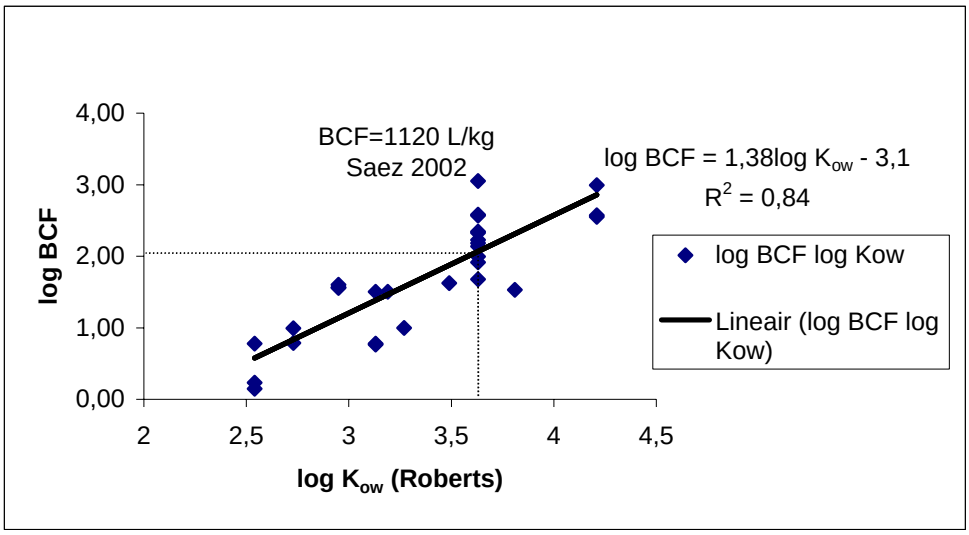

Fig.7.1 Correlation between useful BCF values of single LAS components (Table 4.1 and 4.2) and reported log Kow values estimated by Roberts et al 1991.

Tolls et al (ETC 2000B) showed that the variation of the log BCF is correlated *positively* with the number of CH2 groups by a constant factor of 0.34 (and thus the BCF itself by a factor of 2.2) and *negatively* correlated by the number of ethoxylates by factor of 0.21 (corresponding to a factor of 1.6 in BCF-value). Such a type of correlation is a strong indication that hydrophobic interactions dominate. However the scope of the reported QSAR is small, in other words, the QSAR is only reliable for interpolation rather than extrapolation. The quality of the reported SAR is for each set of criteria as follows: training set 0.80, Method 0.52, Statistics 0.15 (see Annex 4 for the different criteria). The statistical quality is low owing to the lack of validation. This is caused by the small training set. Therefore estimated BCF values in this case will be quickly outside the scope of the QSAR increasing the (random) prediction error of the value rapidly. The QSAR was used to estimate the BCF of a commercial mixture of AEO described by Evans et al (1994) and resulted in a BCF of 142 L/kg.

Rosen et al (2001) studied the relationship between the interfacial properties of surfactants and their toxicity to aquatic organisms and discussed a correlation between the BCF values of the Channel catfish published later (Versteeg 2003) for different LAS components. As independent variable they used the free enthalpy change of the micellisation process (from the Critical Micelle Concentration, the CMC value) and A_{min} the minimum cross-sectional area of the surfactant at the interface ($-\Delta G^0_{ad}/A_{min}$ (mJ/m^2). The correlations between the log BCF values at each specific nominal exposure were reported to be high but were established on a mere 4 values each. They concluded from their work that surfactant toxicity is primarily determined both by its (hydrophobic) adsorption tendency and the ease of its penetration into the cell membrane.

We come here to the important conclusion that at least for LAS and AEO the hydrophobic interaction dominates the BCF . It is expected that this type of interaction prevails for other surfactants as well. This is indirectly shown in the publication of Rosen et al (2001) for cationic surfactants (see section 7.1.2). Despite the experimental problems it is still important to establish descriptors for surfactants that are related to their hydrophobicity. Therefore we report briefly the log K_{ow} estimation methods and use these methods in correlations attempts with the set of useful BCF values from Table 4.1 and 4.2.

7.3 log K_{ow} estimation methods applied to surfactants

The K_{ow} values that can be estimated in the HOCNF standard form refer to the method from Hansch and Leo. However, the contribution of the relevant surfactant fragments is often not defined in the method of Hansch and Leo, leading for e.g for LAS to similar log K_{ow} for different LAS homologues. Several attempts have been published in the literature to derive the relevant fragment contributions and to estimate log K_{ow} values of surfactants. In general the estimated log K_{ow} values obtained in this way are correlated successfully with ecotoxic LC50 and CMCs for all types of surfactants except the amphoteric ones owing to the general lack of data. We have not investigated thoroughly the different estimation methods since this was outside the scope of this assignment, but we report the main findings.

Roberts (1989) refined the fragment method of Hansch and Leo for LAS by including a branch factor. Roberts (1991) extended his method to branched APEO and AEO, used the estimated log K_{ow}-values in this case for correlations with LC50 and CMC values, and obtained reasonable results. Recently he also extended the fragment method of cationic surfactants and obtained good correlations with their CMC and acute aquatic toxicities (Roberts 2003). We have not analysed the quality of these reported correlations but we support his conclusion that this analysis indicates that problems of calculating log K_{ow} of surfactants can be overcome *e.g.* by applying a proper position-dependent branch factor. However the good results on using such a factor must still be regarded as indirect evidence. Roberts' suggestion to use the CMC as a hydrophobic descriptor returns in reported correlations of Cheng et al (2005).

For the AEO, Cheng et al (2005) also reports correlations between log BCF data and estimated log K_{ow}-values. Their log K_{ow}–values are estimated from the UNIFAC method after introducing the ethoxy functional group from vapor-liquid equilibrium data based on small alcohol ethoxylates. One should note however that the deviation of the model calculations from the measured log K_{ow} values for the selected AEO increases substantially upon increasing both the hydrophobic and ethoxylate chain lengths (Table 3 Cheng et al 2005). The method of Cheng was used to estimate the log Kow of the AEO set of BCF values given in a review by Madsen et al (2001). The data set however included 8 direct and 4 indirect BCF values reported by Tolls et al (1994) that were derived without considering the biotransformed fraction. These last ones are therefore overestimations of their true BCF. Therefore we conclude here that the BCF data set has not been composed correctly. The low quality of this correlation is reflected in its evaluation scores (training set: 0.38, method 0.55, statistics 0.00).

We used the BCF data for AEO from the dataset in Table 4.1, together with the log K_{ow}-values from the estimation method from Cheng et al (2005) and from the KOWWIN (vs 1.66) estimation method from EPISUITE that can be downloaded free of charge from the EPA website. One of the differences between the log K_{ow} protocol from Roberts (1991) and the methods from Cheng and KOWWIN is the contribution of the EO-group. Roberts used a value of – 0.10 whereas Cheng uses a value of – 0.36 for his new method based on the UNIFAC contribution model, and the KOWWIN estimates a value of – 0.25. Fig 7.2 shows

the correlation equations. The one based on the Roberts (1991) method is not shown here since the contributions of some relevant fragments are missing.

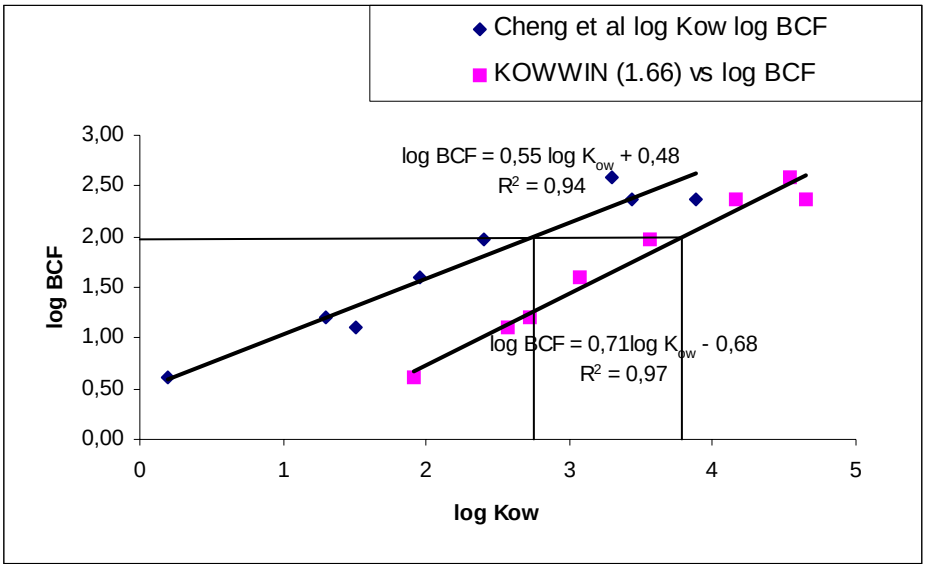

Fig 7.2 Correlation of useful AEO BCF values AEO (from Table 4.1) and their estimated log K_{ow} values according to Cheng et al (2005) and KOWWIN (vs 1.66). Estimated log K_{ow} from Roberts (1991) could not be used because the contribution of the relevant molecular fragments of the AEO could not be derived in this ref.

Note the rather high correlation coefficients in both estimation methods. Note as well that the log K_{ow} values corresponding to a certain BCF value are substantially lower than in the correlation plot of log BCF and log K_{ow} estimated from Roberts (see Fig 7.1) for LAS. The different log K_{ow} estimation methods leads in all cases to high correaltions. However, if one would like to use an estimation method to establish a limit value, the choice of the estimation method leads to differences in substances that for one method wil pass the limit value but not in the other method. In addition the problem with the cutoff value for potential bioaccumulation of log K_{ow} > 3 compared to log BCF > 2 can be clearly seen here. In the estimation method of Cheng et al both cutoff values correspond nicely with each other. Therefore no difference is expected. This is different for KOWWIN where the log K_{ow} cutoff value corresponds to a log BCF_{exp} of 1.40 and thus more AEO are potentially bioaccumulating than that they are bioaccumulating in reality. Using the KOWWIN estimation method, just as Roberts' method, would lead to exclusion of more surfactants (as they would be classified as environmentally hazardous according to the EU Dangerous Substance Directive, DSD 67/548/ECC) than using the Cheng method.

In the paper of Rosen et al (2001) on the relationship between the interfacial properties of surfactants and their toxicity to aquatic organisms the authors determined a number of (Langmuir) adsorption constants (K_L) of a solid immobilised artificial membrane (IAM) of a monolayer of phosphatidyl choline covalently bound to HPLC grade silica via an aminopropyl link and aqueous solutions of several cationic surfactants (C_8-C_{16} TMAC). The Langmuir adsorption parameters, K_L and C_s^{max} were transformed into the independent variable of $-\Delta^l_s G^0_{ad}/^l_s A_{min}$ (mJ/m^2) where A_{min} is is the minimum cross-sectional area of the surfactant at the interface. To obtain A_{min} from C^s_{max} it is necessary that the specific area of the sorbent is determined or in case of a CMC determination the covered surface of a specific quantity of surfactant. Although these experiments are relatively simple, they introduce extra work and costs. The authors obtained a high correlation between the $-\Delta^l_s G^0_{ad}/^l_s A_{min}$ and the number of CH_2 groups of the TMAC used. However, they attributed this directly to the presence of a

hydrophobic interaction in the sorption process. That this conclusion is at least questionable has been explained in our previous report (Krop & de Voogt 2006) but it may turn out well since the variation is mainly caused by the increase of the entropy with increasing number of CH_2-groups. This increase is similar for both the hydrophobic and electrostatic interaction in case the relative enthalpy change (ΔH^0) is small. The authors used the descriptor ($-\Delta^l_s G^0_{ad}/^l_s A_{min}$) to show high correlations with the CMC and EC50-values of the selected cationic surfactans and several organisms. As indicated before the appearance of ΔG^0 in these correlations is due to taking the logarithm of the equilibrium constant. The advantage of including A_{min} in the descriptor is most likely that a high correlation is obtained between all aquatic toxicity values for cationic, anionic and nonionic surfactants for the *rotifer* and ($-\Delta^l_s G^0_{ad}/^l_s A_{min}$). In addition they showed that the descriptor $-\Delta^l_s G^0_{ad}/^l_s A_{min}$ can be replaced by $-\Delta G^0_{ad}/A_{min}$ (mJ/m^2) without losing much of the correlation. This last descriptor is much easier to determine by simple CMC measurement of the specific surfactant.

Concluding, it seems advisable to investigate all published log K_{ow} correlations with surfactant properties like the log CMC, log BCF, -log LC50, etcetera, for the different types of surfactants. By evaluating the contributions from all relevant fragments in these relationships it may be possible to identify fragment values that are successful for predicting relevant endpoints that are hydrophobic in character, like the CMC and BCF. A log K_{ow} estimation method that employs these optimum fragment values could then be applied to surfactants.

7.4 Determination of experimental K_{ow} values

The conclusion from the current literature that the nature of the underlying bioconcentration process is hydrophobic in character explains the search for hydrophobic descriptors like the log K_{ow}. Although it is difficult to determine a K_{ow} of a surfactant directly using the shake flask method (OECD 107), other indirect methods need to be investigated more closely, such as the HPLC method according to OECD 117. The purpose of these methods is to establish a hydrophobic descriptor that can be used in correlations with established BCF values. Information provided in both method protocols (107 and 117) states that they cannot be used for surface-active substances. No further explanation for this exclusion is provided in the information The OECD 117 uses an apolar column (reverse-phase packing) and it is expected that the hydrophobic interaction mechanisms will govern the retention of the substance in the colunm provided the concentration of the surfactant in the mobile phase is sufficiently below its CMC. The exclusion stated in the protocol information has resulted in the search for alternative chromatographic methods. We report briefly some suggestions here. Surfactants may interact in an electrostatic or hydrophobic way with a surface. If one would like to use a chromatographic method, it should be based upon the hydrophobic interaction of the surfactant with the column. Unfortunately we think that Micellar electrokinetic capillary chromatography (MECC), the Microemulsion Electrokinetic Capillary Chromatography (MEEKC) and possibly the micellar electrokinetic chromatography (MEKC) as suggested by EOSCA (2000) seem to be less suitable since the retention of the surfactant in these methods is likely to be influenced by electrostatic interactions. This might not be the case using the common HPLC method (where a reversed phase column is used to ensure that the hydrophobic interactions prevail). The OECD 117 prescribes the employment of an isocratic elution and to include substances with a known log K_{ow} value. However, it is expected that the electrostatic interaction of the head will influence the retention time and to correct this influence it is suggested to vary the gradient of the mobile phase whereby it is possible to derive the value in the limit to 100% water. In this way capacity factors, *k*, are determined for surfactants in 100 % water.

(The same chromatographic method may also be employed to establish a retention time with a strong polar column to estimate the electrostatic interaction of the surfactant and attempt to correlate the derived retention times to sorption constants of surfactant derived from infinite dilution since these are also electrostatic in character).
The established capacity factors cannot be correlated with the log K_{ow} of surfactants since the latter cannot be established by any direct method. However, these capacity factors could be correlated to measured BCF values. In that case a proper set of BCF values needs to be determined for a relevant marine organism (that would allow to account for biotransformation). In this way a cut-off capacity factor could be determined corresponding to a BCF of 100 L/kg (or alternatively 500 L/kg). The use of the HPLC column method has the experimental advantage that it is a well-known and validated method.
Direct log K_{ow} measurements of surfactants according to the slow-stirring method (OECD 123) have not been mentioned before. It is unknown whether the slow-stirring method for surfactants suffers from the same drawbacks as the shake-flask method, but since the method is also using a biphasic system of octanol and water one can expect an emulsion to occur.

7.5 BCF dependency on lipid percentage
For strongly hydrophobic substances that in general metabolise very slowly, like PCBs or dioxins, the BCF value depends on the overall percentage of lipid in the organisms. It is expected that surfactants follow the same behaviour. However, despite the fact that the relative variation in log BCF values correspond highly with log K_{ow} values, and hence suggests a hydrophobic interaction, the measured BCF values do not vary with the lipid content in the fathead minnow, as is shown for LAS and AEO at a lipid content between 2 – 10%, and for fluorsurfactants. (Tolls et al 1999A and 2000B, Sáez 2002, Martin et al 2003A). Simple lipid normalization of BCF values for LAS is therefore not appropriate.

The BCF value of sodium laurate in the zebra fish of 255 L/kg reported by van Egmond (1999) is surprisingly high. Although a correction has been made for the radioactive degradation products in the water phase, this has not been done so in the fish itself. The authors reported that lauric acid was rapidly metabolised to more hydrophobic molecules, especially in fish that survived to day 28. These molecules were analysed in a different extraction phase. Therefore the reported value seems to be an overestimation caused by a different process than for radiolabelled LAS or AEO.

Van Wezel *et al* (1995) have shown that the amount of chemical needed at the target site to produce narcosis is similar for polar and nonpolar narcosis. The distribution of the two classes of contaminants (polar and nonpolar) in different types of lipids does vary, with polar narcosis preferentially partitioning into polar lipids (like surfactants) such as phospholipids (Henderson 1987). In constrast nonpolar narcotics (like PCB, PAH etc) predominate in nonpolar lipids, such as triglycerols and cholesterol. Therefore the different narcotics (surfactants vs PCB, PAH) may partition differently into the different lipids of the organism. However, Tolls *et al*. (1999 and 2000) and Sáez (2002) do not distinguish partitioning of surfactants between different types of lipids in their experiments. The log K_{ow} - log LC50 correlations for anionic, cationic and non-ionic surfactants show that the acute toxicity mechanism corresponds to polar narcosis.

These examples indicate that the distribution of the surfactants in the organism is not as clear as with the strongly hydrophobic substances like PCBs and we report here briefly tissue distribution experiments performed concurrently with a BCF determination. Tolls et al (2000C) displayed a similar pattern in the experimental time course. The C12-2-LAS is being

rapidly taken up by the gills and transported inot systemic circulation and delivered to the liver and other internal organs. They deduced that, given that the liver is the most active organ in xenobiotic transformation and that biotransformation of C12-2-LAS contributes significantly to elimination of the parent LAS, the reduction observed in the liver was most likely brought about by biotransformation, LAS transfer from the gills to the water explais the rapid drop of the gills' concentration ratio.
Newsome et al (1995) determined a number of metabolites of LAS, alkylsulphates and AEOs in several matrices of the goldfish. By far the highest concentrations of the metabolites were determined in the bile matrix. The contrbution of the parent compound to the measured concentrations had decreased signifcantly in a number of cases. For C12-EO3-sulphate the parent contribution accounted for 44%. For the other surfactants it was much lower.
Knezovich et al (1989) indicated that the bioaccumulation of hexadecylpyridinium bromide in clams and minnows were mainly confined to the gills and body. No accumulation was reported for the other internal organs like liver, kidneys etc. This was slightly different for the tadpoles but by far the accumulation after 24h exposure was highest in the gills as well.
Tolls (2000C) concluded that biotransformation of at least C12-2LAS can be seen as a detoxification process, reducing the bioaccumulation potential.

7.6 Comparing BCF mechanism of surfactants and strongly hydrophobic substances like PCB, PAH

The (logarithm of the) uptake rate constants of 7 different LAS (k_1) components in the rainbow trout correlated strongly with the estimated log K_{ow} by Roberts (1989) (Tolls et al 2000) and their depuration rate constants (k_{-1}) did not vary to a great extent. The same variation in the kinetic parameters was seen by Martin *et al* (2003A) for his set of fluorosurfactants. These experiments show the difference in kinetic behaviour between strong hydrophobic substances like PCBs and surfactants. The variation of the (kinetically derived) BCF values (related to hydrophobic parameters) for PCBs depends mainly on the variation of the elimination or depuration process, (k_{-1}), and much less (if at all) on the uptake rate constants. The opposite seems true for anionic, cationic and non-ionic surfactants (Tolls et al 1995). Therefore it does not seem appropriate to compare the BCF behaviour of surfactants with strongly hydrophobic substances like PCB, PAH and dioxins.
Martin *et al* (2003B) describe that perfluorinated alkanoic acids did not accumulate preferentiallly in adipose tissue unlike lipophilic chlorinated organic pollutants. The tissue distribution of PFDA in male rats is similar to their results of the rainbow trout, except that rat liver contained by far the greatest concentrations. Thus the scientific literature indicates that the distribution of the bioaccumulated surfactants over the different organs is different form the strongly lipophilic substances and that the uptake process is determining the bioconcentration process. That this process is related to the hydrophobic character of the surfactant had already been shown by Tolls *et al* (1994).

We conclude from this chapter that:

1. The bioconcentration process of surfactants in fish is hydrophobic in character. Therefore log BCF values do show in general high correlations with hydrophobic descriptors. The absence of such correlations for amphoteric substances is caused by a lack of data. The role of hydrophobic descriptors like K_{ow} or CMC should be analysed more closely.
2. The BCF value of strongly hydrophobic substances depends on the total lipid content. The reported absence of this dependency for surfactants is most likely due to differences in lipid structure in the organism and/or metabolic activities that leads to different partitioning of the surfactants.

3. The search for hydrophobic descriptors that can explain the variation of measured BCF values remains relevant. The log K_{ow} of the surfactant cannot be used as such owing to experimental difficulties. Therefore an indirect experimental method must be used that establish a hydrophobic descriptor. It is suggested to use the chromatographic column method (OECD 117) and to adjust the mobile phase into a gradient approach in such a way that the capacity factor can be derived to a 100% water phase. These capacity factors should then be related to a set of useful BCF values of relevant marine organisms. After standardisation of the column method, the capacity factor is then a measure for the BCF value.
4. Different log K_{ow} estimation methods show different cut-off values that correspond to a BCF of 100 L/kg. Thus the K_{ow} estimation method determines the number of surfactants that may or may not pass the classification scheme. Although the new chemicals policy REACH allows calculation methods in case the log K_{ow} cannot be determined directly, in case of surfactants these methods cannot be compared to experimental values. Since no comparison is possible such methods may not be reliable.

8. Correlation between BCF and K_p data of surfactants

The purpose of this chapter is to conclude whether or not the K_p – values that were qualified as reliable in the report by Krop & de Voogt (2006) can be used for prediction of BCF values of surfactants. To that end we tabulated those BCF values that were qualified as useful or not useful for which reliable Kp values were available. These values are shown in Table 8.1. As the underlying for bioconcentration mechanisms are mainly hydrophobic while sorption mechanisms of surfactants are based mainly on electrostatic interactions, one would expect little, if any, correlation.

Table 8.1 useful sorption constant and both useful and not useful BCF values for common surfactants

Substance name	Type of surfactant	Analytical method	Data analysis	Overall result	Reported * K_p-value (L/kg)	Reported BCF value (L/kg)	English name of species	Ref
C13-EO3	non	**From QSAR Tolls et al (2000B)**			**73**	**309**	fathead minnow	Brownawell 1997
C13-EO6	non	**From QSAR Tolls et al (2000B)**			**11**	**4**	fathead minnow	Brownawell 1997
C13-EO9	non	**From QSAR Tolls et al (2000B)**			**199**	**1**	fathead minnow	Brownawell 1997
C10-in-LAS	an	**Direct (HPLC-fluor)**	**SS**	3	**18**	**3.0**	fathead minnow	Tolls, 1997
C11-in-LAS	an	**Direct (HPLC-fluor)**	**SS**	3	**93**	**9.1**	fathead minnow	Tolls, 1997
C11-LAS	an	**Direct (HPLC-fluor)**	**SS**	2	**74**	**38**	Clam	Saez 2002
C12-in-LAS	an	**Direct (HPLC-fluor)**	**SS**	3	**140**	**29.9**	fathead minnow	
C13-in-LAS	an	**Direct (HPLC-fluor)**	**SS**	3	**2115**	**112.5**	fathead minnow	Tolls, 1997
NPEO2.8	non	**Direct (HPLC-fluor)**	**SS**	2	**230,0**	**4000,0**	Clam	Saez 2002
14C-C12-AS	an	**indirect LSC**	**kinetic**	#	**85**	**4.3**	Proterorhinus marmoratus	
						Not useful BCF values (L/kg)		
14C-C16/18-TMAC	cat	**indirect LSC**	**kinetic**	#	**120 000**	**1962**	fathead minnnow	
14C-C16/18-TMAC	cat	**indirect LSC**	**kinetic**	#		141	fathead minnnow	Selected in Tolls 1994
14C-C12-TMAC	cat	**indirect LSC**	**kinetic**	#	**9 000**	**35**	fathead minnnow	Selected in Tolls 1994
14C-C12-TMAC	cat	**indirect LSC**	**kinetic**	#		41	fathead minnnow	Selected in Tolls 1994

* Reported in Krop& de Voogt 2006.

Corresponding useful BCF and K_p values of AEO were not found. Therefore the QSAR reported by Tolls et al (2000A) was used to estimate the BCF values for those AEO for which useful K_p values are reported. We used the reported BCF values of the different Alkyl-TMACs in the review of Tolls et al (1994) determined in DOC free water. Four indirect BCF values of the C12-alkyl sulphate were reported in the literature. Here we tabulate the average BCF value of 4.3 L/kg. The full data set comprises of 14 surfactants with corresponding BCF and K_p values. A plot of the 9 useful BCF data vs useful K_p values is shown in Fig 7.1.

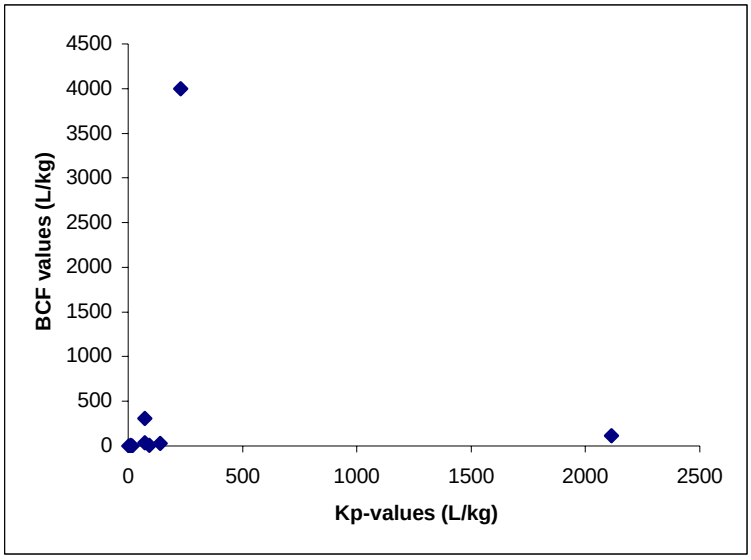

Fig 8.3 Plot of useful BCF values and reported useful K_p-values. Data are from Table 7.1

Table 8.1 shows that there is a tendency that the BCF increases with increasing sorption constant, there is no sign that they are correlated. This tendency holds for the indirect BCF values as well. As indictaed above, the fact that a correlation cannot be expected is explained easily. The BCF process of surfactants is driven by hydrophobic interactions while sorption of surfactants under environmental conditions is driven by electrostatic interactions. Since these electrostatic interactions are non-linear, the sorption strength varies with varying the concentration. However the overall measured sorption constant is often a combination of both the electrostatic and hydrophobic interactions at higher surfactant concentrations. To separate both types of interaction in the overall sorption isotherm and to derive the relevant parameters requires an approach that has not been developed fully yet in the scientific literature.

The CHARM modeling suite uses a K_p estimation method related to the total fraction released. The origin of this estimation method has not been explicated in the CHARM manual. Table 8.2 lists the default K_p values for several classes of surfactants in CHARM.

Table 8.2 *Default values used in the CHARM Hazard Assessment module to estimate K_p values (according to Eq 26c from the CHARM manual version 1.4) for a f_{oc} = 0.04 from the fraction of surfactants released.*

Type of surfactant	**Fraction released, f_r**	**K_p (for f_{oc} = 0.04)**	**Reported K_p**
Quaternary amines	1.0	0.04	C12-C18 -TMACs (9000 – 120000)
EO-PO Block polymer demulsifier (Ethoxylate-Propoxylate)	0.4	10	
Imidazolines	0.1	159	
Fatty amines	0.1	159	
Fatty amides	1.0	0.04	
Primary amines(cationic type, C≥12))	0.1	159	
Phosphate esters (anionic type, C≥13)	0.1	159	
Others	1.0	159	

Comparing the quarternary cationic values from Table 8.1 and 8.2 reveals that the estimation of K_p in CHARM is around a factor of 1 000 (!) out of range with the experimental values. This is likely due to the fact that the fraction relaeased (f_r) is describing a process that is not governed by the K_p. Equation 26c should therefore be reconsidered.

9 **Implications for the assessment of surfactants by the HMCS and the CHARM models**

In this chapter we discuss some implications of the results from this report for the HMCS and CHARM models.

9. 1 BCF values for surfactants used in the offshore industry

Offshore industry has to submit a number of relevant environmental data of chemicals used at the production platforms. One of these is the bioaccumulation potential which is thought to be represented by the log K_{ow} value, established according to the OECD 117 or 107. Since the log K_{ow} of a surfactant cannot be established, the alternative is to use an experimental BCF. As a first step BCF values reported in the scientific literature for surfactants are evaluated. This evaluation leads to a set of useful BCF that can be compared to the list of surfactants used in the offshore industry. The Dutch and Danish lists of surfactants amount to a total of 83 different surfactants mixtures (Krop and de Voogt, 2006). Of these 45 are nonionic, 12 anionic, 13 cationic, 2 amphoteric and 11 surfactants could not be categorised. Nearly all of these surfactants are commercial mixtures although in chemicals policy they may be regarded as a single substance. For around 20% (15 out of 83) of the surfactants on the Dutch and Danish list the reported BCF values could be classified as useful (Table 4.1). Several of these are above the limit BCF value of 100 L/kg (section 4.2.2). These values have been determined in fresh water test systems. For only three surfactants one or more BCF values have been reported that refer to a marine system (Table 4.2). One may conclude that for a substantial number of surfactants (useful) BCF values are missing. Most of the commercial mixtures are chemically unidentified (28 out of 83). At least in those cases the necessary endpoints need to be measured experimentally.

9.2 Correlation between the BCF and sorption constants of surfactants

The relationship between BCF and sorption constants was evaluated for possible predictive purposes. As expected, such a relationship was not found (Chapter 8). As explained in Ch. 7 and 8, the sorption characteristics of a surfactant are a complex function of the electrostatic interactions of the surfactant's polar headgroup and the hydrophobic interactions of the tail with the sediment surface. Under environmentally relevant concentrations in the marine compartment the electrostatic interactions determine the sorption process whereas the hydrophobic interactions determine the bioconcentration process. Thus the BCF and K_p express fundamentally different interactions.

9.3. Bioconcentration of surfactants – process and determination

The bioconcentration process of at least the anionic, cationic and non-ionic surfactants is hydrophobic in character. Therefore experimentally determined BCF values are expected to correlate with hydrophobic parameters like the K_{ow}, and do so indeed. However, contrary to what is expected, BCF values of surfactants do not correlate well with the total lipid content of the organism. The most likely cause is the partitioning character of the surfactant over the different compartments in the organism and the simultaneously occurring biotransformation.

To determine the BCF of a surfactant from the Danish or Dutch lists three problems need to be solved; 1) how to obtain a BCF of a mixture of chemicals that is regarded as a single substance in chemicals policy, 2) to account for the variation in BCF between different species owing to biotransformation and 3) to extrapolate from BCF determined in freshwater test systems to those relevann in marine water.

1) The BCF of a mixture can be obtained by summing the fraction of the BCF of each homologue present in the mixture. Therefore the BCF of a single substance in chemical policy that is composed of different chemical substances can be determined.
2) The BCF of a surfactant depends on the (fish) species used in the experiment. In order to avoid that species are used that do not represent the marine environment, it is advisable to list fish or other marine organisms to be used in BCF experiments or in case they are listed as is normally the case for a standard experiment, to know the influence of biotransformation on the listed species. Most likely invertebrates have lower biotransformation rates than fish and therefore will result in higher BCF value for surfactants. Application of invertebrate BCF data is likely to reduce the number of surfactants that meet the bioaccumulation potential threshold set in the HMCS.
3) It is not known yet whether it is possible to extrapolate BCF values of river water organisms to marine ones, as is possible for sorption constants. Lack of BCF values determined in both water systems that also account for the influence of biotransformation is the main cause of this knowledge gap.

9.4 Log K_{ow} and BCF in chemicals policy

9.4.1 Influence of EU chemicals policy and the HCMS

The fact that the bioconcentration process of surfactants is indeed governed by hydrophobicity makes the search for hydrophobic descriptors relevant. These descriptors are used to derive QSARs in order to estimate the BCF of unknown substances. There are three fundamental problems in deriving such relationships:
I) Bioaccumulation is one of the three endpoints used for environmental hazard classification; the other two being the (bio)degradation and acute aquatic toxicity. It is, however, the potential for bioaccumulation expressed as the log K_{ow} that is primarily used. The present K_{ow} estimation programs of e.g. Roberts and KOWWIN can be used for this purpose since they establish high correlations between log Kow values on the one hand and other hydrophobic endpoints of surfactants, like the CMC and acute toxicity. However these estimation methods pose a classification problem because quite a number of surfactants will possess an estimated log K_{ow} exceeding the limit value of 3.As a consequence these surfactants will be classified as environmentally hazardous (R53) if their acute aquatic toxicity is below 10 mg/L. The log K_{ow} limit value of 3 is originally based on hydrophobic substances that in general show (very) limited biotransformation in the selected fish species. As indicated in this report biotransformation does occur in most of the surfactants used in the offshore and hence reduces the BCF. The previously derived BCF limit value of 100 L/kg corresponding to the limit value for log K_{ow} of 3 would in reality expected to be lower. However this lowering effect is not considered in the log K_{ow} estimation methods. Therefore these estimation methods will not be used on a wide scale in chemicals policy.
It is important to note that the new chemicals policy REACH allows the use of estimation methods of the necessary endpoints for registration (see Chapter 9.4.2). The GHS increases the limit value for log K_{ow} from 3 to 4 (and for the BCF_{exp} from 100 to 500 L/kg (total wet weight)).

II) There are also conceptual problems in the estimation methods for log K_{ow} of surfactants. The problems in the estimation methodologies themselves are caused by the lack of reliable values of the contribution of the relevant molecular fragments of the surfactants, e.g. the contribution of an ethoxylate unit. This contribution cannot be established since experimental log K_{ow} values are lacking, simply because the different experimental standard methods are unsuitable. Therefore one cannot establish a reliable log K_{ow} estimation method for surfactants.

However several log K_{ow} estimation methods were successful because they established good correlations with other hydrophobic parameters like the CMC, acute toxicity and the BCF as well. Since the bioconcentration process of surfactants is indeed determined by hydrophobic interactions it is worthwhile to review these log K_{ow} estimation methods for surfactants as indicated before. In addition, the reported problems in the experimental method for determining log K_{ow} need to be resolved by searching for alternative experimental methods that can establish a hydrophobic parameter that can be used for correlation purposes both with existing log K_{ow} of chemical classes and estimated log K_{ow} methods for e.g. surfactants. Certain chromatographic methods look promising to derive a hydrophobic parameter. It should be possible to adapt OECD 117 in such a way that the isocratic elution is changed by an elution scheme that can derive the capacity factor as hydrophobic parameter of the surfactant to 100% water. These capacity factors cannot be compared to experimental log K_{ow} values but they can be compared to a set of reliable BCF values. Although a set of reliable BCF should be determined, at the far end one can use the capacity factor to determine the BCF value.

III) The BCF test is in general much more expensive than a "simple" K_{ow} test or a chromatographic test to establish a capacity factor of the unknown surfactant. In their direct impact assessment study on REACH (KPMG 2004), TNO quotes a price of € 53 000 for a BCF experiment using one aquatic species. Not only is the standard experiment itself expensive but also the analysis of the surfactants is difficult and may lead to an increase in the overall price, unless one uses the more simple overall analysis of the radiolabelled surfactant. Compare this price to a normal log K_{ow} determination (that cannot be found in the KPMG report) by e.g. *BfB lab* in Belgium of € 3280 for the OECD 107 or € 2650 for the OECD 117 (July 2004) and the price difference is substantial. However it is not necessary to determine (or calculate) either the BCF or the log K_{ow} value in present EU chemicals policy for existing substances and therefore the supplier will not determine either of the two. The financial burden remains then on the user.

9.4.2 Recent changes in chemicals policy

Two large changes in chemicals policy are due to occur.
First, from the 1st of June 2007 the new EU chemicals policy under the acronym REACH will be introduced. It covers a registration of around 30 000 of the 100 000 so-called "existing substances", mainly those with an EINECS number, in 11 years. For all substances that are produced above 1 ton/year/company, the n-octanol-water partition coefficient must be provided. The regulation includes specifically that if the test cannot be performed a calculated value and particulars of the calculation methods must be given. Therefore the establishment of an adequate log K_{ow} calculation method for surfactants seems to be quite relevant.
Second, from the 2010 the GHS will be introduced as well. This will change the current classification and labelling system. Although the system is basically quite similar to the EU one, there are some fundamental differences. One is the difference in communication on the hazards. The R- and S-phrases will be substituted by a signal word and precautionary statement and new labels will appear. In addition some limit values will also change, including the values of log K_{ow} and the BCF for environmental classification. The values will increase for log K_{ow} from 3 to 4 and for an experimental BCF from 100 L/kg to 500 L/kg (total wet weight).

9.5 (Modelling the) behaviour of surfactants in (marine) waters

The HMCS incorporates three aspects: a) The HOCNF that establishes a set of relevant substance data b) a pre-screening phase and 3) a hazard assessment using the environmental fate model CHARM.

Ultimately the aim is to understand and model the environmental fate of surfactants. Currently the fate model CHARM is based only on the hydrophobic interaction of substances with biota and sediment. Therefore the K_{ow} is the key parameter in CHARM.

Including only hydrophobic interaction is not fully correct for surfactants especially in marine sediments. These sediments in general contain quite low organic carbon fractions. Since surfactants may exhibit both hydrophobic and electrostatic interactions the overall interaction between a surfactant and sediment may be higher than expected, rendering a higher than expected sediment and a lower marine water concentration. This may influence the PEC/NEC ratios both in water and sediment that are calculated by CHARM. This is quite relevant for surfactants because sorption isotherms of surfactants with sediment (and soil) with low organic carbon fraction are non linear. Since this aspect is not covered in any fate model, including CHARM, the model does not predict adequately the Hazard or Risk Quotients of surfactants.

It might be possible to derive the relevant mathematical equations describing both interaction processes adequately. However, this will introduce additional parameter(s) in the equations that may not be easily available. Yet, if one would like to include a more realistic method to calculate an hazard or risk quotient of surfactants, one of the first steps is to include the electrostatic interaction parameter relevant for sorption of surfactants. The relevant environmental concentrations of surfactants in the marine compartment are in the range that the electrostatic interaction prevails over the hydrophobic one.

10. Conclusions and recommendations

10.1 Conclusions

Nearly all BCF values for surfactants are determined in freshwater systems. There is substantial lack of useful BCF values determined in marine water organisms.
A total set of 257 BCF values could be retrieved from the literature. 54 % refer to anionic, 31% to cationic and 15 % to nonionic values. From the 54% anionic, 95% referred to LAS compounds and from the 31 % cationic 85% of the BCF values were retrieved from 1 reference of low quality. No BCF values have been reported for amphoteric surfactants.

From a total set of 257 BCF values of surfactants 53 (21%) are of sufficient quality. These 53 BCF values consist of 8 AEO, 29 LAS and 7 perflurorinated surfactant values in fresh water systems. Several BCF values are for the same LAS homologue. Nine values from 3 different surfactants are related to only one marine species.

A group of 6 (classes of) surfactants possesses reported BCF that are either below or above the limit value of 100 L/kg.

Approximately 20% of the Dutch and Danish lists of surfactants are covered by reported BCF values.

The majority of reported experimental BCF values have been obtained from a single fish species, viz. the fathead minnow. The selection of the test organism is critical for the outcome of a BCF experiments. Vertebrates usually have larger biotransformation capacities than invertebrates. It has been shown that surfactants are rapidly biotransformed by several fish species, with the exception of Channel catfish, whereas in several invertebrates (e.g. Hyalella, midge) biotransformation was absent.

The BCF of a surfactant composed of a mixture of homologues can be determined by summing the contributions of the fractions of the individual components and their corresponding BCF.

The experimentally obtained BCF values of surfactants depend on the feeding behaviour of and the biotransformation in organisms. It is not yet fully known whether in general the BCF of surfactants do depend on the exposure concentration and DOC.

The BCF generally increases with increasing mol mass, but at high molecular masses this relationshipdoes not seem to hold. However upper limits that have been set in the past do have been shown to be invalid.
A simple algorithm to estimate the BCF in seawater from a BCF in freshwater cannot be given due to a lack of (useful) BCF data of seawater organisms.

The observed absence of BCF dependency on lipid weight for surfactants is most likely due to a combination of biotransformation and differences in lipid structures that lead to differences in partitioning between surfactants and hydrophobic substances like PCBs.

A correlation between useful BCF and (reliable) K_p could not be established. This is expected since the sorption mechanism in the marine compartment is determined by electrostatic interactions for environmental relevant concentrations contrary to the BCF that is determined by hydrophobic interactions.

Hydrophobic interactions of surfactants are dominant in the bioconcentration process. This is shown in high correlations of CMC, acute toxicity and BCFs of cationic, anionic and non-ionic surfactants with hydrophobic parameters.

To use the log K_{ow} as an indicator of hydrophobicity for surfactants is not possible because the log K_{ow} cannot be established directly owing to experimental problems. However the capacity factor established by column experiments can be used as an indicator for hydrophobicity if the hydrophobic interaction between the surfactant and the column is present (apolar column is used) and the mobile phase is varied in such a way that the capacity factor can be derived for 100% water.

10.2 Recommendations

<u>For the HMCS</u>

The BCF of a surfactant composed of a mixture of different homologues can be obtained by adding the relative contribution of each individual. However, a limit value of the mass fraction considered should be agreed upon (e.g. 95% of the molar fraction of the mixture).

Since the log K_{ow} cannot be determined for surfactants it is necessary to define another hydrophobic indicator and to use this indicator to establish a correlation with a set of reliable BCF values. It is recommended to use the capacity factor from chromatographic experiments on the condition that the chromatographic column mimics the hydrophobic interaction of the surfactant with the column material and that the mobile phase is varied in such a way that the capacity factor can be derived for a 100% water phase. Such is possible by adapting the OECD 117. It is expected that standardization of such a method is quite possible.
As a next step a relevant species should be selected and a set of reliable BCF values should be established possibly for each type of surfactant. The variation in BCF values can then be correlated with the hydrophobic parameter established by the column method. The hydrophobic parameter from the column method is then an indicator for the BCF value that can be used in the HMCS.

To evaluate the existing log K_{ow} estimation methods for surfactants and to select one that leads to good correlations with other hydrophobic parameters and with the few existing experimental log K_{ow} values of surfactants. One has to keep in mind that this approach is in line with the new chemicals policy that will enter into force in phases from 1st of June 2007. In the case an estimation method is selected, for those surfactants that have an estimated log K_{ow} above the limit value set in the HMCS, a BCF experiment should be conducted.

For CHARM

The log K_{ow} is not an appropriate descriptor to estimate a reliable sediment water partition coefficient for surfactants since the interaction of the surfactant with the sediment is not hydrophobic in character anymore at environmentally relevant concentrations. One of the main causes is the low organic carbon fraction of sea sediment. The proposed default K_p values in CHARM, which are based on a default fraction of surfactants released arenot adequate because these are not derived from a sorption model. However the method to incorporate the different types of interactions (electrostatic and hydrophobic) is not described fully in the scientific literature. In addition new parameters will most likely be defined. This makes it necessary to introduce new default values for sediment parameters and/or a specific way to estimate its value.

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Annex 1

Kinetic scheme including an equilibrium and a transformation process

Bioconcentration experiments measure the concentration increase of the specific chemical in the organism (or specific parts of it) over time. For a successful experiment it is necessary that after a specific period the concentration remains the same.
To determine the BCF value of the specific component for the organism two methods are used. The kinetic method uses a non-linear approach by estimating the ratio of the uptake and elimination rate constants using the full exposure curve. The steady state approach uses the steady state concentrations in both phases. In this case it is necessary to determine that steady state can be applied. It has been observed that BCF values for a group of LAS components based on the kinetic model is constantly higher than based on the steady state approximation (Tolls et al 1997). Therefore we will discuss shortly the different kinetic schemes.

Bioconcentration and biotransformation in its most simple form can be described by the following kinetic equation:

$$A \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} B \xrightarrow{k_2} C$$

For biotransformation A and B are chemically identical but are present in different matrices, A being in the water phase and B in the organism. C, the biotransformed product, is chemically different from A or B.

Such schemes are solved in most chemically kinetic textbooks. We are interested in the solution of B while maintaining the concentration of A constant as in a flow-through experiment.

The solution of the concentration of B and time is given by equation A1 (e.g. Connors 1991 is followed here)

$$[B]_t = \frac{k_1[A]^0}{\beta - \alpha}[e^{-\alpha t} - e^{-\beta t}] \qquad \text{(A1)}$$

where α and β are not elementary rate constants: instead they are composite quantities defined by the following equations where

$$\alpha\beta = k_1 k_2$$

$$\alpha + \beta = k_1 + k_{-1} + k_2$$

The exact solution of the variation of the concentration of A and B, C_A and C_B, which is what is the way to determine the BCF_{kin} is given by equation A2 which can be compared to the equilibrium assumption, Eq A3

$$\frac{C_A}{C_B} = k_1\left[\frac{\exp(-\alpha t) - \exp(-\beta t)}{(\beta - k_1)\exp(-\alpha t) - (\alpha - k_1)\exp(-\beta t)}\right] \qquad \text{(A2)}$$

$$\frac{C_A}{C_B} = \frac{k_1}{k_{-1}} \qquad \text{(A3)}$$

According to the OECD 305 Eq A3 should be used to determine the BCF_{kin}. However this is only correct in case or no or very little biotransformation takes place as in experiments with PCBs. For surfactants this is most likely only valid for a number of fluorinated ones and not for most commonly used surfactants. Therefore we conclude here that determination of the BCF_{kin} by the kinetic equation of A_3 does not lead to a correct BCF. For surfactants with biotransformation BCF determined by the steady state method are only appropriate.

However, BCF literature of surfactants does not report such an approach although Tolls et al (ETC 2000) report the quantification of biotranformation of C12-2-LAS in fathead minnows in a slightly different manner.

There are three classes of practical behaviour, as defined by the following conditions:

1) Steady state $k_{-1} + k_2 >> k_1$

2) Preequilibrium $k_1 >> k_2 \text{ AND } k_{-1} >> k_2$

3) Both steady state and Preequilibrium $k_{-1} + k_2 >> k_1 \quad \text{AND} \quad k_1 >> k_2 \text{ AND } k_{-1} >> k_2$

Fig 1A shows the different curves with arbitrary rate constants including the one where no biotransformation takes place.

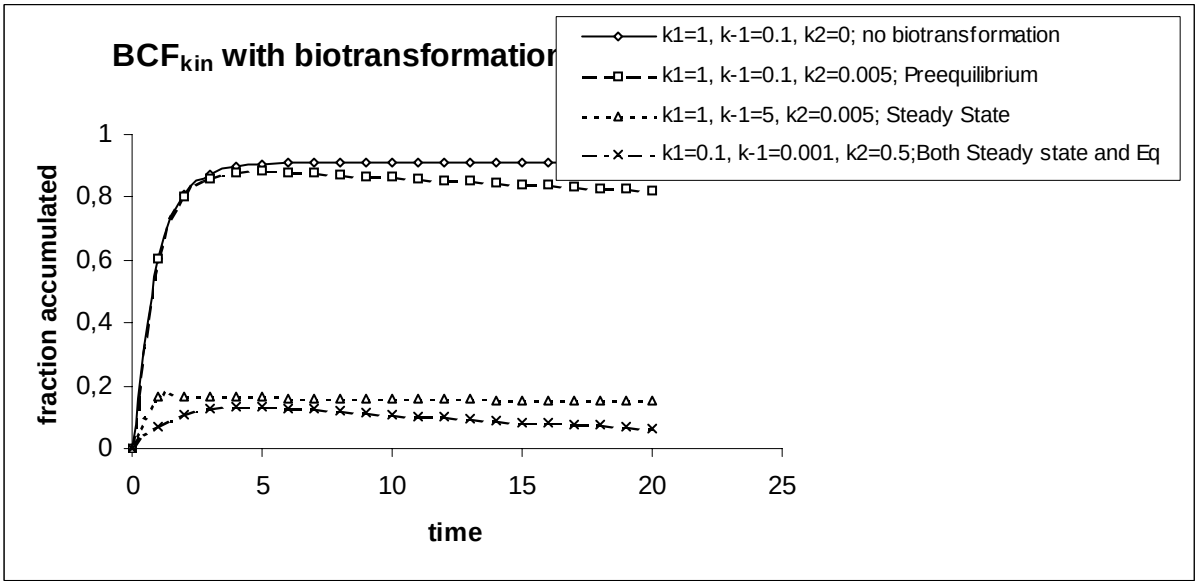

Fig 1A Comparison of the concentration variation of A under the different kinetic conditions as described in the text. Rate constants are indicated.

Fig 1A shows that when the BCF_{kin} is determined according to the method described in the OECD 305 it is assumed that the preequilibrium curve is valid. This curve is nearly equivalent to the curve when no biotransformation has taken place. The variation in both curves is insignificant compared to the experimental error. However, if biotransformation is significant as is the case for biodegradable surfactants, then the lower curves are expected in a kinetic experiment. Fig 1A shows that the curves according to the OECD 305 (preequilibrium) are steeper at the origin than the curves when biotransformation is significant. Therefore it is expected and observed that a BCF_{kin} in the first case is higher than in the second case. It is however the second one that gives the correct value which is the same as when the BCF is

determined in the steady state range based on the ratio of the concentration, C_A^{ss}/C_B^{ss}. This difference in BCF-values is also observed by Tolls et al (1997). It is rather astonishing to note that no reference uses the correct kinetic scheme in case the BCF_{kin} is determined. In that case one would obtain all three kinetic parameters in one correlation.

In this approach the following important observations can be made in relation to experimental determined BCF values (BCF):

- The BCF value in an organism depends on the biotransformation rate constants, k_2. These are different for each organism. Therefore a substance BCF cannot be defined. The choice of the organism influences heavily the measured BCF value.
- BCF values determined by the steady state method are preferred.
- BCF values determined by the (pre)equilibrium kinetic method are expected to be an overestimation of the correct BCF value. Therefore scientifically useful BCF values should be determined by the steady state method if the correct kinetic scheme is not used. However the kinetically determined BCF values are useful in case the BCF is limited in screening schemes like the HCONS. If below the limit value the correct BCF will still remain below the limit value.

Annex 2

Criteria to assess the BCF reference

FIRST AUTHOR: ..... **YEAR:.....Journal:……**

General analytical criteria.

	Sco.	Wt.
a) Has the analysis being performed by an established standard method? (e.g. recommended by the OECD)?	..	3
b) Is the stated recovery sufficiently high? (state.....)	..	5
c) 1) Has the linear dynamic range been established?	..	1
or 2) Is the measured value within the linear dynamic range?	..	3
and Is the detection limit of the substance in wate and the fish tissues stated?	..	3
d) Are product interferences absent?	..	4
e) Is correction applied for the dead time of the radiation detector?	..	4
	____	(20/22)

General methodic criteria.

a) Is the determination of the parameter the main aim of the study	..	1
b) Is an appropriate method used (e.g. recognised by the OECD)?	..	3
c) Is the method correctly applied within its limits?	..	5
d) Is, if appropriate, the experiment performed below the maximum aqueous solubility of the substance?	..	5
e) Has the temperature been kept constant sufficiently during the experiment?	..	4
f) Is the purity of the used substances sufficiently high?	..	3
g) Has the identity of the used substances been checked for?	..	2
h) Is corrected for a control and/or blanc experiment?	..	5
i) If appropriate, is the correct mass balance determined during the experiment?	..	5
j) If appropriate, Is the measured value of the parameter of the radioactive labelled substance not significantly different from its non-radioactive one?	..	4
k) Is the experiment being carried out by a single substance?	..	3
	____	(40)

Specific methodic criteria.

a) Were the organisms cultivated by a reported standard procedure?	..	3
b) Are the test organisms exposed to uncontaminated water before the experiment for at least 48 hours?	..	3
c) Was the highest concentration of the contaminant less than 1/10 of the LC-50 value of the test organism and at least 10 fold higher than its detection limit in water?	..	4
d) Has the preparation of the test-solution being sufficiently described?	..	3
e) Is pH and [O_2(aq)] sufficiently kept constant during the experiment?	..	3
f) Is the organism being exposed to day and night rhythm, if appropriate?	..	2
g) If appropriate, is the biomass of the system (0.1 – 1.0 g fish(wet weight)/L water) such that the kinetic assumptions are satisfied?	..	4
h) Is the water concentration not exhausted during the experiment?	..	5
i) If appropriate, is the flow velocity at least 5 x volume of the aquarium/24 hour?	..	3
j) If appropriate, is the used co-solvent not toxic and not degradable?	..	4
k) Has the extraction and clean up of the test-organism been performed by an official standardized method?	..	4
l) Has the test-compound been removed regularly from the solution		

during depuration ? .. 2
m) If appropriate, was corrected for third phase interference .. 5
n) If appropriate, is the tissue percent of solids reported? .. 2
o) Is TOC (not passing a 0.45 μm filter) below 5 mg/L during the experiment? .. 1

____ (48)

General statistic criteria.

a) Does the accuracy of the measured parameter (C.I.) agree with the kind of experiment?
   1) CI < 20%) .. 5/5
   2) CI 20 – 30% .. 3/5
   3) CI 30 – 50% .. 1/5
   4) CI > 50% .. 0/5

b) Are a sufficient number (>3) of replicates being analysed for each measuring point? .. 3
c) Is the number of data at each concentration point larger than 2? .. 2
d) Were sufficient measurement points taken in line with the kind of experiment? .. 5
e)
   1)Is equilibrium or steady state sufficiently proven (at least 1/3 of the time period of the experiment with four measurements?) .. 5
   2) Is the number of measurements sufficient when using the rate constant model? (5,5) .. 4
f) Was measured at different concentration with a difference of at least a factor of 10? .. 2
g) Is the regression coefficient sufficiently high, when fitting the curve? .. 4

_____ (34)

Results:

SUBSTANCE:	Analytic	Methodic	Statistic	Completeness
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....

Annex 3

Reported BCF values

The following table shows all the reported BCF values of surfactants with additional information and their score. The information in the necessary supporting fields are given here.

The **substance name** is the name of the substance as it appears in the reference. Since refrenrences do not cite CAS RN, CAS numbers are not indicated here. If a radioactive surfactant was used the name of the surfactant starts with the radioactive atom. C12-2-LAS means a LAS with an linear alkyl chain of 12 atoms and on the 2-position the p-sulfophenyl group is attached.
The **type of surfactant** is divided into cationic, anionic, non-ionic and amphoteric. However, ammonium-related surfactants can either be positively charged as an ammonium salt or be neutral as an amine-compound. Both behave as a cationic surfactant.
The **analytical method** is either direct or indirect. In case it is direct, the analytical method is indicated briefly, like GC-FID; the substance is identified by gas chromatography using a FID detector.
Data-analysis is the way the data are analysed in the BCF determination. The following options are given. Kinetic if the reported BCF is a BCF_{kin}; steady state if the reported BCF is a BCF_{ss};. If the BCF has been determined by the steady state method but steady state was not deterined, e.g in field experiment, it is idicated as SS-n_{SS}
Batch refers to the experimental , BCF Literature reports lab batches, field batches and caged ones in mesocosm experiment. These batches can be subdivided. The variation of the **aquatic concentration in time** is stated here and refers often to distinct the different types of BCF. One can select here a static, semi static or flow-through variation.
The **reported BCF value** in L/kg and **reported statistical parameters** accompanying the reported BCF value. In several cases the substance was below the detection limit in the water or in the fish. In the first case the BCF cannot be determined (nd). In the second case the BCF is actually zero since the substance is not detected in the fish but is in the surrounding water compartment.

The **base** of the BCF value varies in the literature. For small fish the BCF is normally based on the whole body wet weight. However, sometimes the value is based on dry weight. In that case the amount of solids needs to be determined in order to be able to modify dry weight into wet weight. For strongly hydrophobic substances it is common to establish also the BCF value on the lipid weight. In that case the fraction of lipid should be stated as well. In addition in several references the BCF was determined based on the concentration of the substance in a specific tissue. These last values are not suitable for the purpose of this report but are included in the overall database.

The **exposure period**, the **lipid** content and both the Latin and English name of the species are indicated. The refrence, the overall quality of the BCF values as described in chapter 3 and the outcome of the set of criteria applied to the reference are indicated as well. The aqueous concentration (range), the applied ionic strength and the temperature are stated if indicated in the reference in the overall table.

Substance name	type of surfactant	Analytical method	Data analysis	Batch, if indicated	Aq conc time	Reported BCF value (L/kg)	Reported statistical parameters	BCF unit base	Exp period	% lipid	*Latin name of specie*	English name of species	Ref	Overall result	An.	Met	Stat	Aqueous concentration	Ionic strength	T-exp
Laurate (sodium)	an	Direct (LSC, GC-FID)	SS-n_{ss}	lab	flow through	**255**	22 (SD, n=8)	wet weight	28d	n.d	*Danio Rerio*	Zebra fish	Egmond (1999)	**1**	0.54	0.48	0.45	2.0 - 6.4 mg/L (nominal)	96.5 mg/L CaCO3	21.4
Pfoctanoic acid	an	Direct (LC-MS-MS)	kinetic	lab	flow through	**4.0**	ser=0.60, N=1	wet weight (carcass)	12d/33d	nd	*Oncorhynchus mykiss*	Rainbow trout	Martin, ETC 2003 A	**2**	0.42	0.55	0.50	1E-3 μg/L	unknown	unknown
Pfdecanoic acid	an	Direct (LC-MS-MS)	kinetic	lab	flow through	**450**	ser=62, N=1	wet weight (carcass)	12d/33d	nd	*Oncorhynchus mykiss*	Rainbow trout	Martin, ETC 2003 A	**3**	0.42	0.55	0.70	8E-4 μg/L	unknown	unknown
Pfundecanoic acid	an	Direct (LC-MS-MS)	kinetic	lab	flow through	**2700**	ser=400, M=1	wet weight (carcass)	12d/33d	nd	*Oncorhynchus mykiss*	Rainbow trout	Martin, ETC 2003 A	**3**	0.42	0.55	0.70	6E-4 μg/L	unknown	unknown
Pfdodecanoic acid	an	Direct (LC-MS-MS)	kinetic	lab	flow through	**18000**	ser=2700, N=1	wet weight (carcass)	12d/33d	nd	*Oncorhynchus mykiss*	Rainbow trout	Martin, ETC 2003 A	**3**	0.42	0.55	0.70	2E-4 μg/L	unknown	unknown
Pftetradecanoic acid	an	Direct (LC-MS-MS)	kinetic	lab	flow through	**23000**	ser=5300, N=1	wet weight (carcass)	12d/33d	nd	*Oncorhynchus mykiss*	Rainbow trout	Martin, ETC 2003 A	**2**	0.42	0.55	0.50	2E-5 μg/L	unknown	unknown
Pfoctane sulfonate	an	Direct (LC-MS-MS)	kinetic	lab	flow through	**1100**	ser=150, N=1	wet weight (carcass)	12d/33d	nd	*Oncorhynchus mykiss*	Rainbow trout	Martin, ETC 2003 A	**3**	0.42	0.55	0.70	2E-3 μg/L	unknown	unknown
Pfhexanesulfonate	an	Direct (LC-MS-MS)	kinetic	lab	flow through	**9.6**	ser=0.99, N=1	wet weight (carcass)	12d/33d	nd	*Oncorhynchus mykiss*	Rainbow trout	Martin, ETC 2003 A	**3**	0.42	0.55	0.70	5E-3 μg/L	unknown	unknown
Pfoctane sulfonate	an	Direct (LC-MS/MS)	SS-n_{ss}	field	#	**8850**	none	liver tissue	not rel	n.d.		small- + largemouth bass	Sinclair 2006	**1**	0,83	not rel	0,12	3 - 7 ng/L	not rel	not rel
C10-2-LAS	an	Direct (RP-HPLC-fluor)	SS	lab	flow through	**1.4**	0.6 (std, n=6)	wet weight	120 h	?	*Oncorhynchus mykiss*	Rainbow trout	Tolls 2000B	**2**	0,53	0,57	0,3	1327 nM	[Me2+]= 1.21 mM	14
C11-2-LAS	an	Direct (RP-HPLC-fluor)	SS	lab	flow through	**6**	1 (std, n=6)	wet weight	120 h	?	*Oncorhynchus mykiss*	Rainbow trout	Tolls 2000B	**2**	0,53	0,57	0,3	622 nM	[Me2+]= 1.21 mM	14
C12-2-LAS	an	Direct (RP-HPLC-fluor)	SS	lab	flow through	**82**	8 (std, n=6)	wet weight	120 h	?	*Oncorhynchus mykiss*	Rainbow trout	Tolls 2000B	**2**	0,53	0,57	0,3	195 nM	[Me2+]= 1.21 mM	14
C13-2-LAS	an	Direct (RP-HPLC-fluor)	SS	lab	flow through	**372**	45 (std, n=6)	wet weight	120 h	?	*Oncorhynchus mykiss*	Rainbow trout	Tolls 2000B	**2**	0,53	0,57	0,3	75 nM	[Me2+]= 1.21 mM	14
C12-2-LAS	an	Direct (HPLC-fluor)	SS-n_{ss}	lab-A	flow through	**47.6**	rsd = 26%	wet weight	48 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997	**1**	0.44	0.59	0.38	<4.1 μM	art fresh water	22
C10-2-LAS	an	Direct (HPLC-fluor)	SS-n_{ss}	lab-A	flow through	**1.7**	rsd = 29%	wet weight	48 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997	**1**	0.44	0.59	0.38	<4.1 μM	art fresh water	22
C11-2-LAS	an	Direct (HPLC-fluor)	SS-n_{ss}	lab-A	flow through	**5.8**	rsd = 27%	wet weight	48 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997	**1**	0.44	0.59	0.38	<4.1 μM	art fresh water	22
C13-2-LAS	an	Direct (HPLC-fluor)	SS-n_{ss}	lab-A	flow through	**353.8**	not stated	wet weight	48 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997	**1**	0.44	0.59	0.38	<4.1 μM	art fresh water	22

C12-2-LAS	an					**153**	CV=9%	wet weight					Tolls, 2000	Overall value		0.44	0.63	0.58	not dependent on aq conc	art fresh water	
C12-2-LAS	an	Direct (HPLC-fluor)	SS	lab-B	flow through	**99.1**	rsd = 19%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C11-5-LAS	an	Direct (HPLC-fluor)	SS	lab-B	flow through	**6.1**	rsd = 49%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C12-5-LAS	an	Direct (HPLC-fluor)	SS	lab-B	flow through	**10.0**	rsd = 44%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C13-5-LAS	an	Direct (HPLC-fluor)	SS	lab-B	flow through	**34.0**	rsd = 34%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C12-2-LAS	an	Direct (HPLC-fluor)	SS	lab-C	flow through	**168.4**	rsd = 37%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C11-5-LAS	an	Direct (HPLC-fluor)	SS	lab-C	flow through	**9.8**	rsd = 53%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C12-6-LAS	an	Direct (HPLC-fluor)	SS	lab-C	flow through	**31.9**	rsd = 48%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C12-3-LAS	an	Direct (HPLC-fluor)	SS	lab-C	flow through	**42.1**	rsd = 42 %	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C12-2-LAS	an	Direct (HPLC-fluor)	SS	lab-D	flow through	**211.5**	rsd = 27%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C10-2-LAS	an	Direct (HPLC-fluor)	SS	lab-D	flow through	**6.0**	rsd = 46%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C11-2-LAS	an	Direct (HPLC-fluor)	SS	lab-D	flow through	**31.9**	rsd = 29%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C13-2-LAS	an	Direct (HPLC-fluor)	SS	lab-D	flow through	**987.2**	rsd = 22%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C10-in-LAS	an	Direct (HPLC-fluor)	SS	lab-D	flow through	**3.0**	rsd = 50%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C11-in-LAS	an	Direct (HPLC-fluor)	SS	lab-D	flow through	**9.1**	rsd = 41%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C12-in-LAS	an	Direct (HPLC-fluor)	SS	lab-D	flow through	**29.9**	rsd = 27%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C13-in-LAS	an	Direct (HPLC-fluor)	SS	lab-D	flow through	**112.5**	rsd = 28%	wet weight	196 h	2-10%	*Pimephales promelas*	fathead minnow	Tolls, 1997		**3**	0.44	0.63	0.58	<4.1 μM	art fresh water	22
C12-2-LAS	an	Direct (HPLC-fluor)	SS	lab-1	flow through	**222**	33 (std, n=9)	wet weight	168 h		*Pimephales promelas*	fathead minnow	Tolls 2000A		**3**	0,44	0,59	0,77	0.104 μM nominal	art fresh water	22
C12-2-LAS	an	Direct (HPLC-fluor)	SS	lab-2	flow through	**138**	37 (std, n=20)	wet weight	165 h		*Pimephales promelas*	fathead minnow	Tolls 2000A		**2**	0,44	0,59	0,32	0.194 μM nominal	art fresh water	22
14C-C12-2-LAS	an	Direct (LSC/TLC)	kinetic	lab	semi static	**240**	80 (95% CI)	wet weight (feeding not allowed in exp vessel)	10d uptake/5d elim		*Chironomu riparius*	Midge	Hwang 2003		**2**	0.58	0.53	0.45	0.0045 mg/L	standard hard water	20
14C-C12-2-LAS	an	Direct (LSC/TLC)	SS	lab	semi static	**39**		wet weight (feeding allowed in exp vessel)	4d and 12 d		*Chironomu riparius*	Midge	Hwang 2003		**2**	0.58	0.53	0.45	0.037 - 1.72 mg/L	standard hard water	20
C12-LAS	an	direct(HPLC/M	SS-n_{ss}	caged	flow	**104**	no data	wet weight	32 days	n.d.	*Ictalarys*	channel	Versteeg		**1**	0.40	0.45	0.09	0.13 mg/L	320 μS	18

		S)			through						*punctatus*	catfish	2003										
		direct(HPLC/M			flow						*Ictalarys*	channel	Versteeg										
C12-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**72**	no data	wet weight	32 days	n.d.	*punctatus*	catfish	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18
		direct(HPLC/M			flow						*Ictalarys*	channel	Versteeg										
C12-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**42**	no data	wet weight	32 days	n.d.	*punctatus*	catfish	2003	**1**	0.40	0.50	0.09	0.93 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-2-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**150**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-2-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**165**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-2-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**185**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-3-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**35**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-3-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**50**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-3-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**60**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-4-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**20**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-4-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**30**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-4-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**35**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-5-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**25**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-5-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**35**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18
		direct(HPLC/M			flow							fathead	Versteeg										
C12-5-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**35**	no data	wet weight	32 days	n.d.		minnow	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-2-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**95**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-2-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**65**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-2-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**130**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-3-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**60**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-3-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**30**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-3-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**120**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-4-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**55**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-4-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**30**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18
		direct(HPLC/M			flow								Versteeg										
C12-4-LAS	an	S)	$SS\text{-}n_{ss}$	caged	through	**125**	no data	wet weight	32 days	n.d.	*Hyella azteca*		2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18
C12-5-LAS	an	direct(HPLC/M	$SS\text{-}n_{ss}$	caged	flow	**45**	no data	wet weight	32 days	n.d.	*Hyella azteca*		Versteeg	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18

		S)			through							2003								
		direct(HPLC/M			flow							Versteeg								
C12-5-LAS	an	S)	SS-n_{ss}	caged	through	**15**	no data	wet weight	32 days	n.d.	*Hyella azteca*	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-5-LAS	an	S)	SS-n_{ss}	caged	through	**70**	no data	wet weight	32 days	n.d.	*Hyella azteca*	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-2-LAS	an	S)	SS-n_{ss}	caged	through	**20**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.45	0.09	2.97 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-2-LAS	an	S)	SS-n_{ss}	caged	through	**45**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-2-LAS	an	S)	SS-n_{ss}	caged	through	**45**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-2-LAS	an	S)	SS-n_{ss}	caged	through	**60**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-3-LAS	an	S)	SS-n_{ss}	caged	through	**5**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.45	0.09	2.97 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-3-LAS	an	S)	SS-n_{ss}	caged	through	**15**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-3-LAS	an	S)	SS-n_{ss}	caged	through	**20**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-3-LAS	an	S)	SS-n_{ss}	caged	through	**25**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-4-LAS	an	S)	SS-n_{ss}	caged	through	**3**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.45	0.09	2.97 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-4-LAS	an	S)	SS-n_{ss}	caged	through	**8**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-4-LAS	an	S)	SS-n_{ss}	caged	through	**8**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-4-LAS	an	S)	SS-n_{ss}	caged	through	**20**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-5-LAS	an	S)	SS-n_{ss}	caged	through	**3**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.45	0.09	2.97 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-5-LAS	an	S)	SS-n_{ss}	caged	through	**8**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-5-LAS	an	S)	SS-n_{ss}	caged	through	**10**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-5-LAS	an	S)	SS-n_{ss}	caged	through	**15**	no data	wet weight	32 days	n.d.	*Corbicula*	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-2-LAS	an	S)	SS-n_{ss}	caged	through	**20**	no data	wet weight	32 days	n.d.	*Elimia*	2003	**1**	0.40	0.45	0.09	2.97 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-2-LAS	an	S)	SS-n_{ss}	caged	through	**35**	no data	wet weight	32 days	n.d.	*Elimia*	2003	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-2-LAS	an	S)	SS-n_{ss}	caged	through	**60**	no data	wet weight	32 days	n.d.	*Elimia*	2003	**1**	0.40	0.50	0.09	0.29 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-2-LAS	an	S)	SS-n_{ss}	caged	through	**70**	no data	wet weight	32 days	n.d.	*Elimia*	2003	**1**	0.40	0.50	0.09	0.13 mg/L	320 µS	18	
		direct(HPLC/M			flow							Versteeg								
C12-3-LAS	an	S)	SS-n_{ss}	caged	through	**10**	no data	wet weight	32 days	n.d.	*Elimia*	2003	**1**	0.40	0.45	0.09	2.97 mg/L	320 µS	18	
C12-3-LAS	an	direct(HPLC/M	SS-n_{ss}	caged	flow	**10**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg	**1**	0.40	0.45	0.09	0.93 mg/L	320 µS	18	

		S)			through							2003									
C12-3-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**30**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.50	0.09	0.29 mg/L	320 μS	18
C12-3-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**50**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.50	0.09	0.13 mg/L	320 μS	18
C12-4-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**5**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.45	0.09	2.97 mg/L	320 μS	18
C12-4-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**10**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.45	0.09	0.93 mg/L	320 μS	18
C12-4-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**20**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.50	0.09	0.29 mg/L	320 μS	18
C12-4-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**20**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.50	0.09	0.13 mg/L	320 μS	18
C12-5-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**5**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.45	0.09	2.97 mg/L	320 μS	18
C12-5-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**15**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.45	0.09	0.93 mg/L	320 μS	18
C12-5-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**20**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.50	0.09	0.29 mg/L	320 μS	18
C12-5-LAS	an	direct(HPLC/MS)	SS-n_{ss}	caged	flow through	**15**	no data	wet weight	32 days	n.d.	*Elimia*	Versteeg 2003		**1**	0.40	0.50	0.09	0.13 mg/L	320 μS	18
14C-C12-LAS	an	indirect LSC	kinetic			**108**		wet weight			*Lepomis macrochirus*	Selected in Tolls 1994	Bishop 1980	**2**	#	#	#	0.23 μM		
14C-C12-LAS	an	indirect LSC	kinetic			**145**		wet weight			*Lepomis macrochirus*	Selected in Tolls 1994	Bishop 1980	**2**	#	#	#	0.23 μM		
14C-C12-LAS	an	indirect LSC	kinetic			**227**		wet weight			*Lepomis macrochirus*	Selected in Tolls 1994	Bishop 1980	**2**	#	#	#	2.3 μM		
14C-C12-LAS	an	indirect LSC	kinetic			**280**		wet weight			*Lepomis macrochirus*	Selected in Tolls 1994	Bishop 1980	**2**	#	#	#	2.3 μM		
14C-C12-LAS	an	indirect LSC	SS-n_{ss}			**173**		wet weight			*Pimephales promelas*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	0.30 μM		
14C-C12-LAS	an	indirect LSC	SS-n_{ss}			**245**		wet weight			*Pimephales promelas*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	0.40 μM		
14C-C12-LAS	an	indirect LSC	SS-n_{ss}			**551**		dry weight			*Pimephales promelas*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	0.29 μM		
14C-C12-LAS	an	indirect LSC	kinetic			**231**		wet weight			*Brachydanio Rerio*	Selected in Tolls 1994	Coenen 1988	**2**	#	#	#	0.30 μM		
14C-C12-LAS	an	indirect LSC	SS-n_{ss}			**490**		dry weight			*Daphnia magna*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	0.20 μM		

14C-C12-LAS	an	indirect LSC	SS-n_{ss}	**560**	dry weight	*Daphnia magna*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	0.32 μM
14C-C12-LAS	an	indirect LSC	SS-n_{ss}	**720**	dry weight	*Daphnia magna*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	1.26 μM
14C-C12-LAS	an	indirect LSC	SS-n_{ss}	**8**	wet weight	*Daphnia magna*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	0.20 μM
14C-C12-LAS	an	indirect LSC	SS-n_{ss}	**58**	wet weight	*Daphnia magna*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	0.46 μM
14C-C12-LAS	an	indirect LSC	SS-n_{ss}	**103**	wet weight	*Daphnia magna*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	1.26 μM
14C-C13-LAS	an	indirect LSC	SS-n_{ss}	**385**	wet weight	*Pimephales promelas*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	0.28 μM
14C-C13-LAS	an	indirect LSC	SS-n_{ss}	**293**	wet weight	*Pimephales promelas*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	0.31 μM
14C-C13-LAS	an	indirect LSC	SS-n_{ss}	**1223**	dry weight	*Pimephales promelas*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	0.28 μM
14C-C13-LAS	an	indirect LSC	SS-n_{ss}	**142**	wet weight	*Daphnia magna*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	0.25 μM
14C-C13-LAS	an	indirect LSC	SS-n_{ss}	**240**	wet weight	*Daphnia magna*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	1.13 μM
14C-C13-LAS	an	indirect LSC	SS-n_{ss}	**1250**	dry weight	*Daphnia magna*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	0.25 μM
14C-C13-LAS	an	indirect LSC	SS-n_{ss}	**1050**	dry weight	*Daphnia magna*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	0.30 μM
14C-C13-LAS	an	indirect LSC	SS-n_{ss}	**1325**	dry weight	*Daphnia magna*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	1.13 μM
14C-C11.6-LAS	an	indirect LSC	SS-n_{ss}	**269**	dry weight	*Pimephales promelas*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	0.79 μM
14C-C11.6-LAS	an	indirect LSC	SS-n_{ss}	**50**	wet weight	*Pimephales promelas*	Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	2.63 μM
14C-C11.6-LAS	an	indirect LSC	SS-n_{ss}	**480**	dry weight	*Daphnia magna*	Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	2.68 μM

14C-C11.7-LAS	an	indirect LSC	SS-n_{ss}			**104**	wet weight			*Lepomis macrochirus*		Selected in Tolls 1994	Kimerle 1981	**1**	#	#	#	1.45 μM								
14C-C13.1-LAS	an	indirect LSC	SS-n_{ss}			**472**	dry weight			*Pimephales promelas*		Selected in Tolls 1994	Cmotto 1979	**1**	#	#	#	1.17 μM								
14C-C13.1-LAS	an	indirect LSC	SS-n_{ss}			**4100**	dry weight			*Daphnia magna*		Selected in Tolls 1994	Comotto 1979	**1**	#	#	#	2.53 μM								
14C-C13.1-LAS	an	indirect LSC	SS-n_{ss}			**696**	wet weight			*Daphnia magna*		Selected in Tolls 1994	Kimerle 1975	**1**	#	#	#	2.52 μM								
14C-C12-AS	an	indirect LSC	kinetic			**7.15**	wet weight			*Proterorhinus marmoratus*		Selected in Tolls 1994	Topcuoglu 1981	**1**	#	#	#	13.9 μM								
14C-C12-AS	an	indirect LSC	kinetic			**2.7**	wet weight			*Cyprinus Carpio*		Selected in Tolls 1994	Wakabayashi 1091	**1**	#	#	#	0.093 μM								
14C-C12-AS	an	indirect LSC	kinetic			**4.6**	wet weight			*Cyprinus Carpio*		Selected in Tolls 1994	Wakabayashi 1091	**1**	#	#	#	1.39 μM								
14C-C12-AS	an	indirect LSC	kinetic			**2.6**	wet weight			*Cyprinus Carpio*		Selected in Tolls 1994	Wakabayashi 1091	**1**	#	#	#	13.9 μM								
C11-LAS (74% -3-)	an	Direct HPLC-fluor	SS	lab	flow through	**40**	wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.79	0.14	60 μg/L	sterilised sea water	21.6						
C11-LAS (74% -3-)	an	Direct HPLC-fluor	SS	lab	flow through	**36**	wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.79	0.14	190 μg/L	sterilised sea water	21.6						
C11-LAS (74% -3-)	an	Direct HPLC-fluor	SS	lab	flow through	**37**	wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.79	0.14	350 μg/L	sterilised sea water	21.6						
C12-2-LAS	an	Direct HPLC-fluor	SS	lab	flow through	**1120**	wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.74	0.14	30 μg/L	sterilised sea water	17.2						
C12-2-LAS	an	Direct HPLC-fluor	SS	lab	flow through	**370**	wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.74	0.14	100 μg/L	sterilised sea water	17.2						
C12-2-LAS	an	Direct HPLC-fluor	SS	lab	flow through	**380**	wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.74	0.14	140 μg/L	sterilised sea water	17.2						
C12-2-LAS	an	Direct HPLC-fluor	SS	lab	flow through	**1900**	gills	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.74	0.14	30 μg/L	sterilised sea water	17.2						
C12-2-LAS	an	Direct HPLC-fluor	SS	lab	flow through	**750**	gills	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.74	0.14	100 μg/L	sterilised sea water	17.2						
C12-2-LAS	an	Direct HPLC-	SS	lab	flow	**1870**	digestive	5 d	1,4 -	*Ruditapes*	Clam	Saez		**2**	0.66	0.74	0.14	30 μg/L	sterilised	17.2						

		fluor			through			gland		2,0	*semidecussatus*		2002					sea water	
C12-2-LAS	an	Direct HPLC-fluor	SS	lab	flow through	**2130**		digestive gland	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002	**2**	0.66	0.74	0.14	100 μg/L	sterilised sea water	17.2
CH3CH3N(+)(C10H21)(C10H21) DDAC	cat	?? (LSC)	??		??	**81**	??	wet weight	??		*Pimephales Promelas*	Fathead Minnow	In Jurgeuns en 2000	**?**						
C14H29CN	cat	Direct (GC-FID/NPD)	SS	field		**250000**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	0.1 ng/L	sea water	nd
C16H33CN	cat	Direct (GC-FID/NPD)	SS	field		**125000**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	3 ng/L	sea water	nd
C18H37CN	cat	Direct (GC-FID/NPD)	SS	field		**87000**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
C18H35CN	cat	Direct (GC-FID/NPD)	SS	field		**152000**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
CH3NC16H33	cat	Direct (GC-FID/NPD)	SS	field		**5000**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	0.8 ng/L	sea water	nd
CH3NC18H37	cat	Direct (GC-FID/NPD)	SS	field		**10500**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	1.9 ng/L	sea water	nd
CH3N(C16H33)(C16H33)	cat	Direct (GC-FID/NPD)	SS	field		**6800**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	5 ng/L	sea water	nd
CH3N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field		**7000**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	22 ng/L	sea water	nd
CH3N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field		**15000**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	17 ng/L	sea water	nd
C16H33N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field		**nd**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C16H33N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field		**nd**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C18H37N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field		**nd**	no data	fresh weight	n.a.	n.d.	*Capitella capitata*		Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C14H29CN	cat	Direct (GC-FID/NPD)	SS	field		**10000**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*		Valis 1989	**1**	0	n.a.	0	0.1 ng/L	sea water	nd
C16H33CN	cat	Direct (GC-FID/NPD)	SS	field		**1700**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*		Valis 1989	**1**	0	n.a.	0	3 ng/L	sea water	nd
C18H37CN	cat	Direct (GC-FID/NPD)	SS	field		**2000**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*		Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
C18H35CN	cat	Direct (GC-FID/NPD)	SS	field		**4500**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*		Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd

CH3NC16H33	cat	Direct (GC-FID/NPD)	SS	field	**3750**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*	Valis 1989	**1**	0	n.a.	0	0.8 ng/L	sea water	nd
CH3NC18H37	cat	Direct (GC-FID/NPD)	SS	field	**7900**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*	Valis 1989	**1**	0	n.a.	0	1.9 ng/L	sea water	nd
CH3N(C16H33)(C16H33)	cat	Direct (GC-FID/NPD)	SS	field	**9800**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*	Valis 1989	**1**	0	n.a.	0	5 ng/L	sea water	nd
CH3N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**6000**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*	Valis 1989	**1**	0	n.a.	0	22 ng/L	sea water	nd
CH3N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**8700**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*	Valis 1989	**1**	0	n.a.	0	17 ng/L	sea water	nd
C16H33N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C16H33N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C18H37N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*polychaete sp.*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C14H29CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	0.1 ng/L	sea water	nd
C16H33CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	3 ng/L	sea water	nd
C18H37CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
C18H35CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
CH3NC16H33	cat	Direct (GC-FID/NPD)	SS	field	**12500**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	0.8 ng/L	sea water	nd
CH3NC18H37	cat	Direct (GC-FID/NPD)	SS	field	**52000**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	1.9 ng/L	sea water	nd
CH3N(C16H33)(C16H33)	cat	Direct (GC-FID/NPD)	SS	field	**1000**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	5 ng/L	sea water	nd
CH3N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**2600**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	22 ng/L	sea water	nd
CH3N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**13000**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	17 ng/L	sea water	nd
C16H33N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C16H33N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd

C18H37N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Macropipus sp*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C14H29CN	cat	Direct (GC-FID/NPD)	SS	field	**10000**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	0.1 ng/L	sea water	nd
C16H33CN	cat	Direct (GC-FID/NPD)	SS	field	**700**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	3 ng/L	sea water	nd
C18H37CN	cat	Direct (GC-FID/NPD)	SS	field	**1500**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
C18H35CN	cat	Direct (GC-FID/NPD)	SS	field	**2000**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
CH3NC16H33	cat	Direct (GC-FID/NPD)	SS	field	**6250**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	0.8 ng/L	sea water	nd
CH3NC18H37	cat	Direct (GC-FID/NPD)	SS	field	**28000**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	1.9 ng/L	sea water	nd
CH3N(C16H33)(C16H33)	cat	Direct (GC-FID/NPD)	SS	field	**600**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	5 ng/L	sea water	nd
CH3N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**1600**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	22 ng/L	sea water	nd
CH3N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**3600**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	17 ng/L	sea water	nd
C16H33N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C16H33N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C18H37N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Sardinella aurits*	Valis 1989	**1**	0	n.a.	0	< detection limit	sea water	nd
C14H29CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989	**1**	0	n.a.	0	0.1 ng/L	sea water	nd
C16H33CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989	**1**	0	n.a.	0	3 ng/L	sea water	nd
C18H37CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
C18H35CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989	**1**	0	n.a.	0	2 ng/L	sea water	nd
CH3NC16H33	cat	Direct (GC-FID/NPD)	SS	field	**8750**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989	**1**	0	n.a.	0	0.8 ng/L	sea water	nd
CH3NC18H37	cat	Direct (GC-FID/NPD)	SS	field	**34000**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989	**1**	0	n.a.	0	1.9 ng/L	sea water	nd
CH3N(C16H33)(C16H33)	cat	Direct (GC-FID/NPD)	SS	field	**400**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989	**1**	0	n.a.	0	5 ng/L	sea water	nd
CH3N(C16H	cat	Direct (GC-	SS	field	**1500**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis	**1**	0	n.a.	0	22 ng/L	sea	nd

33)(C18H37)		FID/NPD)									1989							water	
CH3N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**4000**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989		**1**	0	n.a.	0	17 ng/L	sea water	nd
C16H33N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989		**1**	0	n.a.	0	< detection limit	sea water	nd
C16H33N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989		**1**	0	n.a.	0	< detection limit	sea water	nd
C18H37N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Sepia officilais*	Valis 1989		**1**	0	n.a.	0	< detection limit	sea water	nd
C14H29CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	0.1 ng/L	sea water	nd
C16H33CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	3 ng/L	sea water	nd
C18H37CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	2 ng/L	sea water	nd
C18H35CN	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	2 ng/L	sea water	nd
CH3NC16H33	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	0.8 ng/L	sea water	nd
CH3NC18H37	cat	Direct (GC-FID/NPD)	SS	field	**0**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	1.9 ng/L	sea water	nd
CH3N(C16H33)(C16H33)	cat	Direct (GC-FID/NPD)	SS	field	**800**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	5 ng/L	sea water	nd
CH3N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**2700**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	22 ng/L	sea water	nd
CH3N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**5500**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	17 ng/L	sea water	nd
C16H33N(C16H33)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	< detection limit	sea water	nd
C16H33N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	< detection limit	sea water	nd
C18H37N(C18H37)(C18H37)	cat	Direct (GC-FID/NPD)	SS	field	**nd**	no data	fresh weight	n.a.	n.d.	*Soles soles*	Valis 1989		**1**	0	n.a.	0	< detection limit	sea water	nd
14C-(C16/18)2-DDAC	cat	indirect LSC	SS-n_{ss}		**32**		wet weight			*Lepomis macrochirus*	Selected in Tolls 1994	Lewis 1983	**1**	#	#	#	0.034 μM		
14C-(C16/18)2-	cat	indirect LSC	SS-n_{ss}		**13**		wet weight			*Lepomis macrochirus*	Selected in Tolls	Lewis 1983	**1**	#	#	#	0.039 μM		

DDAC														1994												
14C-(C18)2-DDAC	cat	indirect LSC	kinetic			**104**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-(C18)2-DDAC	cat	indirect LSC	kinetic			**38**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-(C18)2-DDAC	cat	indirect LSC	kinetic			**3**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-C16/18-TMAC	cat	indirect LSC	kinetic			**1962**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-C16/18-TMAC	cat	indirect LSC	kinetic			**141**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-C12-TMAC	cat	indirect LSC	kinetic			**35**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-C12-TMAC	cat	indirect LSC	kinetic			**41**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-C8-TMAC	cat	indirect LSC	kinetic			**2.4**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-C8-TMAC	cat	indirect LSC	kinetic			**0.5**		wet weight			*Pimephales promelas*			Selected in Tolls 1994	Versteeg 1992	**1**	#	#	#	n.a.						
14C-C13EO8	non	Drect (LSC/TLC)	kinetic		flow through	**31.4**	rsd = 21%	wet weight	33 h	1 - 7%	*Pimephales promelas*	fathead minnow	Tolls, 1999			**3**	0,29	0,69	0,53	0.2 mg/L	art fresh water	22				
C12EO8	non	Direct (HPLC-Flu det)	SS	1	flow through	**12.7**	std 2.8	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A			**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5				
C13EO8	non	Direct (HPLC-Flu det)	SS	1	flow through	**49.9**	std 10.3	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A			**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5				
C13EO4	non	Direct (HPLC-Flu det)	SS	1	flow through	**232.5**	std 55.4	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A			**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5				
C13EO8	non	Direct (HPLC-Flu det)	SS	2	flow through	**25.9**	std 7.4	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A			**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5				
C14EO11	non	Direct (HPLC-Flu det)	SS	2	flow through	**15.8**	std 3.3	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A			**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5				
C14EO8	non	Direct (HPLC-Flu det)	SS	2	flow through	**56.7**	std 15.2	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A			**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5				
C14EO4	non	Direct (HPLC-Flu det)	SS	2	flow through	**237.0**	std 62.3	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A			**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5				
C13EO8	non	Direct (HPLC-Flu det)	SS	3	flow through	**39.5**	std 18.1	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A			**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5				
C14EO14	non	Direct (HPLC-	SS	3	flow	**<5**		wet weight	45h	n.d.	*Pimephales*	fathead	Tolls,			**2**	0,54	0,67	0,31	LBB < 10%	art fresh	20.7 -				

		Flu det)			through						*promelas*	minnow	2000A							water	22.5
C13EO8	non	Direct (HPLC-Flu det)	SS	4	flow through	**55.0**	std 22.4	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A		**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5
C14EO8	non	Direct (HPLC-Flu det)	SS	4	flow through	**135.2**	std 34.3	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A		**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5
C16EO8	non	Direct (HPLC-Flu det)	SS	3+4	flow through	**387.5**	std 313.7	wet weight	45h	n.d.	*Pimephales promelas*	fathead minnow	Tolls, 2000A		**2**	0,54	0,67	0,31	LBB < 10%	art fresh water	20.7 - 22.5
14C-C14-EO7	non	indirect LSC	SS-n_{ss}			**721**		wet weight			*Lepomis macrochirus*		Selected in Tolls 1994	Bishop 1978	**1**	#	#	#	0.41 μM		
14C-C14-EO7	non	indirect LSC	SS-n_{ss}			**731**		wet weight			*Lepomis macrochirus*		Selected in Tolls 1994	Bishop 1978	**1**	#	#	#	0.41 μM		
14C-C14-EO7	non	indirect LSC	SS-n_{ss}			**684**		wet weight			*Lepomis macrochirus*		Selected in Tolls 1994	Bishop 1978	**1**	#	#	#	4.10 μM		
14C-C14-EO7	non	indirect LSC	SS-n_{ss}			**799**		wet weight			*Lepomis macrochirus*		Selected in Tolls 1994	Bishop 1978	**1**	#	#	#	4.10 μM		
14C-C12-EO4	non	indirect LSC	kinetic			**309**		wet weight			*Cyprinus Carpio*		Selected in Tolls 1994	Wakabayashi 1987	**1**	#	#	#	0.69 μM		
14C-C12-EO8	non	indirect LSC	kinetic			**222**		wet weight			*Cyprinus Carpio*		Selected in Tolls 1994	Wakabayashi 1987	**1**	#	#	#	0.45 μM		
14C-C12-EO16	non	indirect LSC	kinetic			**4.3**		wet weight			*Cyprinus Carpio*		Selected in Tolls 1994	Wakabayashi 1987	**1**	#	#	#	0.28 μM		
AE-mix from Evans 1994						**142**	estimated based on compostion from Evans	wet weight					Tolls, 2000A								
NPEO2.8	non	Direct HPLC-fluor	SS	lab	flow through	**4460**		wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.74	0.14	3 μg/L	sterilised sea water	22.3
NPEO2.8	non	Direct HPLC-fluor	SS	lab	flow through	**3700**		wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.74	0.14	4 μg/L	sterilised sea water	22.3
NPEO2.8	non	Direct HPLC-fluor	SS	lab	flow through	**3960**		wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**2**	0.66	0.74	0.14	8 μg/L	sterilised sea water	22.3
NPEO2.8	non	Direct HPLC-fluor	kinetic	lab	flow through	**4390**		wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002		**1**	0.66	0.74	0.14	3 μg/L	sterilised sea water	22.3
NPEO2.8	non	Direct HPLC-fluor	kinetic	lab	flow through	**5690**		wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatu*	Clam	Saez 2002		**1**	0.66	0.74	0.14	4 μg/L	sterilised sea	22.3

NPEO2.8	non	Direct HPLC-fluor	kinetic	lab	flow through	**5420**	wet weight	5 d	1,4 - 2,0	*Ruditapes semidecussatus*	Clam	Saez 2002	**1**	0.66	0.74	0.14	8 µg/L	water sterilised sea water	22.3
NPEO2	non		SS-n_{ss}	field		**37**	muscle tissue			*barbus barbus*		Ahel 93	**0**	#	#	#			
NPEO1	non		SS-n_{ss}	field		**19**	muscle tissue			*barbus barbus*		Ahel 93	**0**	#	#	#			
NPEO2	non		SS-n_{ss}	field		**0.8**	muscle tissue			*Rainbow trout*		Ahel 93	**0**	#	#	#			
NPEO1	non		SS-n_{ss}	field		**3**	muscle tissue			*Oncorhynchus mykiss*	Rainbow trout	Ahel 93	**0**	#	#	#			
NPEO2	non		SS-n_{ss}	field		**2**	muscle tissue			*Squalus cephalus*		Ahel 93	**0**	#	#	#			
NPEO1	non		SS-n_{ss}	field		**1**	muscle tissue			*Squalus cephalus*		Ahel 93	**0**	#	#	#			
NPEO3	non		SS-n_{ss}	caged	flow through	**60**	wet weight			*Mytilus edulis*	mussel	Wahlberg 90	**0**	#	#	#			
NPEO2	non		SS-n_{ss}	caged	flow through	**100**	wet weight			*Mytilus edulis*	mussel	Wahlberg 90	**0**	#	#	#			
NPEO1	non		SS-n_{ss}	caged	flow through	**170**	wet weight			*Mytilus edulis*	mussel	Wahlberg 90	**0**	#	#	#			

Annex 4

QSAR criteria for the determination of the quality of the correaltion

I. ***Criteria for the training set***	***Score***	***Weight***
a) Are the values extracted from primary references?		.. 4
b) Are the values measured by a recommended method (*e.g.* OECD)?		.. 3
c) Is the accuracy of each value stated?		.. 4
d) Are all values of the used reference included? If not, is a proper explanation given?		.. 5
d) Is the number of data points (n) equal to n≥3p+5 (p is the number of descriptors in the model)		.. 4
e) Is any bias in the training set diminished by including values from other primary sources for the same substance?		.. 3
		_____(23)
II. ***Criteria for the selected method***		
a) Do all chosen descriptors possess a mechanistic background?		.. 3
b) Can the mechanism be described by a linear relationship?		.. 4
c) Has the QSAR been applied within its scope, family, range etc.?		.. 3
d) Are the chosen descriptors orthogonal?		.. 3
		_____(13)
III. ***Statistical criteria***		.. 3
a) Has the model been validated properly?		.. 3
b) Has a residual analysis been evaluated?		.. 3
c) Is any under of over fitting of the model prevented?		.. 3
d) Is the prediction interval of the single response stated?		.. 5
e) Are the values of n, r (of r2) and the standard error of regression stated?		.. 3
f) Is it shown that all chosen descriptors are significant		.. 4
		_____(24)
SUBSTANCES Training set Method Statistics Completeness		

Annex 5

On the use of reported BCF in the environmental hazard classification according to the GHS

Table 4.1.1: Classification scheme for substances hazardous to the aquatic environment

Classification criterion elements				Classification categories	
Toxicity		Degradability (*Note 3*)	Bioaccumulation (*Note 4*)		
Acute (*Note 1a and 1b*)	Chronic (*Note 2a and 2b*)			Acute	Chronic
Box 1: value ≤ 1.00 mg/l		**Box 5:**	**Box 6:**	**Category: Acute 1** Box 1	**Category: Chronic 1** Boxes 1+5+6 Boxes 1+5 Boxes 1+6
Box 2: 1.00 < value ≤ 10.0 mg/l		lack of rapid degradability	BCF ≥ 500 or, if absent log K_{ow} ≥ 4	**Category: Acute 2** Box 2	**Category: Chronic 2** Boxes 2+5+6 Boxes 2+5 Boxes 2+6 Unless Box 7
Box 3: 10.0 < value ≤ 100 mg/l				**Category: Acute 3** Box 3	**Category: Chronic 3** Boxes 3+5+6 Boxes 3+5 Boxes 3+6 Unless Box 7
Box 4: No acute toxicity (*Note 5*)	**Box 7:** value > 1.00 mg/l				**Category: Chronic 4** Boxes 4+5+6 Unless Box 7

Notes to Table 4.1.1:

NOTE 1a: *Acute toxicity band based on $L(E)C_{50}$ values in mg/l for fish, crustacea and/or algae or other aquatic plants (or QSAR estimation if no experimental data).*

NOTE 1b: *Where the algal toxicity ErC_{50} [= EC_{50} (growth rate)] falls more than 100 times below the next most sensitive species and results in a classification based solely on this effect, consideration should be given to whether this toxicity is representative of the toxicity to aquatic plants. Where it can be shown that this is not the case, professional judgment should be used in deciding if classification should be applied. Classification should be based on the ErC_{50}. In circumstances where the basis of the EC_{50} is not specified and no ErC_{50} is recorded, classification should be based on the lowest EC_{50} available.*

NOTE 2a: *Chronic toxicity band based on NOEC values in mg/l for fish or crustacea or other recognized measures for long-term toxicity.*

NOTE 2b: *It is the intention that the system be further developed to include chronic toxicity data.*

NOTE 3. *Lack of rapid degradability is based on either a lack of Ready Biodegradability or other evidence of lack of rapid degradation.*

NOTE 4: *Potential to bioaccumulate, based on an experimentally derived BCF ≥ 500 or, if absent, a log K_{ow} ≥ 4 provided log K_{ow} is an appropriate descriptor for the bioaccumulation potential of the substance. Measured log K_{ow} values take precedence over estimated values and measured BCF values take precedence over log K_{ow} values.*

NOTE 5: *"No acute toxicity" is taken to mean that the $L(E)C_{50}$ is above the water solubility. Also for poorly soluble substances, (water solubility < 1.00 mg/l), where there is evidence that the acute test would not have provided a true measure of the intrinsic toxicity.*

A9.5 Bioaccumulation

A9.5.1 *Introduction*

A9.5.1.1 Bioaccumulation is one of the important intrinsic properties of chemical substances that determine the potential environmental hazard. Bioaccumulation of a substance into an organism is not aazard in itself, but bioconcentration and bioaccumulation will result in a body burden, which may or may not lead to toxic effects. In the harmonized integrated hazard classification system for human health and environmental effects of chemical substances (OECD, 1998), the wording "potential for bioaccumulation" is given. A distinction should, however, be drawn between bioconcentration and bioaccumulation. Here bioconcentration is defined as the net result of uptake, transformation, and elimination of a substance in an organism due to waterborne exposure, whereas bioaccumulation includes all routes of exposure (i.e. via air, water, sediment/soil, and food). Finally, biomagnification is defined as accumulation and transfer of substances via the food chain, resulting in an increase of internal concentrations in organisms on higher levels of the trophic chain (European Commission, 1996). For most organic chemicals uptake from water (bioconcentration) is believed to be the predominant route of uptake. Only for very hydrophobic substances does uptake from food becomes important. Also, the harmonized classification criteria use the bioconcentration factor (or the octanol/water partition coefficient) as the measure of the potential for bioaccumulation. For these reasons, the present guidance document only considers bioconcentration and does not discuss uptake via food or other routes.

A9.5.1.2 Classification of a chemical substance is primarily based on its intrinsic properties. However, the degree of bioconcentration also depends on factors such as the degree of bioavailability, the physiology of test organism, maintenance of constant exposure concentration, exposure duration, metabolism inside the body of the target organism and excretion from the body. The interpretation of the bioconcentration potential in a chemical classification context therefore requires an evaluation of the intrinsic properties of the substance, as well as of the experimental conditions under which bioconcentration factor (BCF) has been determined. Based on the guide, a decision scheme for application of bioconcentration data or log Kow data for classification purposes has been developed. The emphasis of the present section is organic substances and organo-metals. Bioaccumulation of metals is also discussed in Section A9.7.

A9.5.1.3 Data on bioconcentration properties of a substance may be available from standardized tests or may be estimated from the structure of the molecule. The interpretation of such bioconcentration data for classification purposes often requires detailed evaluation of test data. In order to facilitate this evaluation two additional appendixes are enclosed. These appendixes describe available methods (Appendix III of Annex 9) and factors influencing the bioconcentration potential (Appendix IV of Annex 9). Finally, a list of standardized experimental methods for determination of bioconcentration and Kow are attached (Appendix V of Annex 9) together with a list of references (Appendix VI of Annex 9).

A9.5.2 *Interpretation of bioconcentration data*

A9.5.2.1 Environmental hazard classification of a chemical substance is normally based on existing data on its environmental properties. Test data will only seldom be produced with the main purpose of facilitating a classification. Often a diverse range of test data is available which does not necessarily match the classification criteria. Consequently, guidance is needed on interpretation of existing test data in the context of hazard classification.

A9.5.2.2 Bioconcentration of an organic substance can be experimentally determined in bioconcentration experiments, during which BCF is measured as the concentration in the organism relative to the concentration in water under steady-state conditions and/or estimated from the uptake rate constant (*k1*) and the elimination rate constant (*k2*) (OECD 305, 1996). In general, the potential of an organic substance to bioconcentrate is primarily related to the lipophilicity of the substance. A measure of lipophilicity is the *n*-octanol-water partition coefficient (Kow), which, for lipophilic non-ionic organic substances, undergoing minimal metabolism or biotransformation within the organism, is correlated with the bioconcentration factor. Therefore, Kow is often used for estimating the bioconcentration of organic

substances, based on the empirical relationship between log BCF and log Kow. For most organic substances, estimation methods are available for calculating the Kow. Data on the bioconcentration properties of a substance may thus be (i) experimentally determined, (ii) estimated from experimentally determined Kow, or (iii) estimated from Kow values derived by use of Quantitative Structure Activity Relationships (QSARs). Guidance for interpretation of such data is given below together with guidance on assessment of chemical classes, which need special attention.

A9.5.2.3 *Bioconcentration factor (BCF)*
A9.5.2.3.1 The bioconcentration factor is defined as the ratio on a weight basis between the concentration of the chemical in biota and the concentration in the surrounding medium, here water, at steady state. BCF can thus be experimentally derived under steady-state conditions, on the basis of measured concentrations. However, BCF can also be calculated as the ratio between the first-order uptake and elimination rate constants; a method which does not require equilibrium conditions.
A9.5.2.3.2 Different test guidelines for the experimental determination of bioconcentration in fish have been documented and adopted, the most generally applied being the OECD test guideline (OECD 305, 1996).

A9.5.2.3.3 Experimentally derived BCF values of high quality are ultimately preferred for classification purposes as such data override surrogate data, e.g. Kow.

A9.5.2.3.4 High quality data are defined as data where the validity criteria for the test method applied are fulfilled and described, e.g. maintenance of constant exposure concentration; oxygen and temperature variations, and documentation that steady-state conditions have been reached, etc. The experiment will be regarded as a high-quality study, if a proper description is provided (e.g. by Good Laboratory Practice (GLP)) allowing verification that validity criteria are fulfilled. In addition, an appropriate analytical method must be used to quantify the chemical and its toxic metabolites in the water and fish tissue (see section 1, Appendix III for further details).

A9.5.2.3.5 BCF values of low or uncertain quality may give a false and too low BCF value; e.g. application of measured concentrations of the test substance in fish and water, but measured after a too short exposure period in which steady-state conditions have not been reached (cf. OECD 306, 1996, regarding estimation of time to equilibrium). Therefore, such data should be carefully evaluated before use and consideration should be given to using Kow instead.

A9.5.2.3.6 If there is no BCF value for fish species, high-quality data on the BCF value for other species may be used (e.g. BCF determined on blue mussel, clam, scallop (ASTM E 1022-94)). Reported BCFs for microalgae should be used with caution.

A9.5.2.3.7 For highly lipophilic substances, e.g. with log Kow above 6, experimentally derived BCF values tend to decrease with increasing log Kow. Conceptual explanations of this non-linearity mainly refer to either reduced membrane permeation kinetics or reduced biotic lipid solubility for large molecules. A low bioavailability and uptake of these substances in the organism will thus occur. Other factors comprise experimental artefacts, such as equilibrium not being reached, reduced bioavailability due to sorption to organic matter in the aqueous phase, and analytical errors. Special care should thus be taken when evaluating experimental data on BCF for highly lipophilic substances as these data will have a much higher level of uncertainty than BCF values determined for less lipophilic substances.
A9.5.2.3.8 BCF in different test species

A9.5.2.3.8.1 BCF values used for classification are based on whole body measurements. As stated previously, the optimal data for classification are BCF values derived using the OECD 305 test method or internationally equivalent methods, which uses small fish. Due to the higher gill surface to weight ratio for smaller organisms than larger organisms, steady-state conditions will be reached sooner in smaller organisms than in larger ones. The size of the organisms (fish) used in bioconcentration studies is thus of considerable importance in relation to the time used in the uptake phase, when the reported BCF value is based solely on measured concentrations in fish and water at steady-state. Thus, if large fish, e.g. adult

salmon, have been used in bioconcentration studies, it should be evaluated whether the uptake period was sufficiently long for steady state to be reached or to allow for a kinetic uptake rate constant to be determined precisely.

A9.5.2.3.8.2 Furthermore, when using existing data for classification, it is possible that the BCF values could be derived from several different fish or other aquatic species (e.g. clams) and for different organs in the fish. Thus, to compare these data to each other and to the criteria, some common basis or normalization will be required. It has been noted that there is a close relationship between the lipid content of a fish or an aquatic organism and the observed BCF value. Therefore, when comparing BCF values across different fish species or when converting BCF values for specific organs to whole body BCFs, the common approach is to express the BCF values on a common lipid content. If e.g. whole body BCF values or BCF values for specific organs are found in the literature, the first step is to calculate the BCF on a % lipid basis using the relative content of fat in the fish (cf. literature/test guideline for typical fat content of the test species) or the organ. In the second step the BCF for the whole body for a typical aquatic organism (i.e. small fish) is calculated assuming a common default lipid content. A default value of 5% is most commonly used (Pedersen *et al.*, 1995) as this represents the average lipid content of the small fish used in OECD 305 (1996).

A9.5.2.3.8.3 Generally, the highest valid BCF value expressed on this common lipid basis is used to determine the wet weight based BCF-value in relation to the cut off value for BCF of 500 of the harmonized classification criteria (see Chapter 4.1, Figure 4.1.1).

A9.5.2.3.9 Use of radiolabelled substances

A9.5.2.3.9.1 The use of radiolabelled test substances can facilitate the analysis of water and fish samples. However, unless combined with a specific analytical method, the total radioactivity measurements potentially reflect the presence of the parent substance as well as possible metabolite(s) and possible metabolized carbon, which have been incorporated in the fish tissue in organic molecules. BCF values determined by use of radiolabelled test substances are therefore normally overestimated.

A9.5.2.3.9.2 When using radiolabelled substances, the labelling is most often placed in the stable part of the molecule, for which reason the measured BCF value includes the BCF of the metabolites. For some substances it is the metabolite which is the most toxic and which has the highest bioconcentration potential. Measurements of the parent substance as well as the metabolites may thus be important for the interpretation of the aquatic hazard (including the bioconcentration potential) of such substances.

A9.5.2.3.9.3 In experiments where radiolabelled substances have been used, high radiolabel concentrations are often found in the gall bladder of fish. This is interpreted to be caused by biotransformation in the liver and subsequently by excretion of metabolites in the gall bladder (Comotto *et al.*, 1979; Wakabayashi *et al.*, 1987; Goodrich *et al.*, 1991; Toshima *et al.*, 1992). When fish do not eat, the content of the gall bladder is not emptied into the gut, and high concentrations of metabolites may build up in the gall bladder. The feeding regime may thus have a pronounced effect on the measured BCF. In the literature many studies are found where radiolabelled compounds are used, and where the fish are not fed. As a result high concentrations of radioactive material are found in the gall bladder. In these studies the bioconcentration may in most cases have been overestimated. Thus when evaluating experiments, in which radiolabelled compounds are used, it is essential to evaluate the feeding regime as well.

A9.5.2.3.9.4 If the BCF in terms of radiolabelled residues is documented to be ≥1000, identification and quantification of degradation products, representing ≥10% of total residues in fish tissues at steady-state, are for e.g. pesticides strongly recommended in the OECD guideline No. 305 (1996). If no identification and quantification of metabolites are available, the assessment of bioconcentration should be based on the measured radiolabelled BCF value. If, for highly bioaccumulative substances (BCF ≥500), only BCFs based on the parent compound and on radiolabelled measurements are available, the latter should thus be used in relation to classification.

A9.5.3 ***Chemical classes that need special attention with respect to BCF and Kow values***

A9.5.3.1 There are certain physico-chemical properties, which can make the determination of BCF or its measurement difficult. These may be substances, which do not bioconcentrate in a manner consistent with their other physico-chemical properties, e.g. steric hindrance or substances which make the use of descriptors inappropriate, e.g. surface activity, which makes both the measurement and use of log Kow inappropriate.

A9.5.3.2 *Difficult substances*

A9.5.3.4 *High molecular weight substances*

Above certain molecular dimensions, the potential of a substance to bioconcentrate decreases. This is possibly due to steric hindrance of the passage of the substance through gill membranes. It has been proposed that a cut-off limit of 700 for the molecular weight could be applied (e.g. European Commission, 1996). However, this cut-off has been subject to criticism and an alternative cut-off of 1000 has been proposed in relation to exclusion of consideration of substances with possible indirect aquatic effects (CSTEE, 1999). In general, bioconcentration of possible metabolites or environmental degradation products of large molecules should be considered. Data on bioconcentration of molecules with a high molecular weight should therefore be carefully evaluated and only be used if such data are considered to be fully valid in respect to both the parent compound and its possible metabolites and environmental degradation products.

A9.5.3.5 *Surface-active agents*

A9.5.3.5.1 Surfactants consist of a lipophilic (most often an alkyl chain) and a hydrophilic part (the polar headgroup). According to the charge of the headgroup, surfactants are subdivided into classes of anionic, cationic, non-ionic, or amphoteric surfactants. Due to the variety of different headgroups, surfactants are a structurally diverse class of compounds, which is defined by surface activity rather than by chemical structure. The bioaccumulation potential of surfactants should thus be considered in relation to the different subclasses (anionic, cationic, non-ionic, or amphoteric) instead of to the group as a whole. Surface-active substances may form emulsions, in which the bioavailability is difficult to ascertain. Micelle formation can result in a change of the bioavailable fraction even when the solutions are apparently formed, thus giving problems in interpretation of the bioaccumulation potential**.**

A9.5.3.5.3 Octanol-water-partition coefficient (Kow)

The octanol-water partition coefficient for surfactants can not be determined using the shakeflask or slow stirring method because of the formation of emulsions. In addition, the surfactant molecules will exist in the water phase almost exclusively as ions, whereas they will have to pair with a counter-ion in order to be dissolved in octanol. Therefore, experimental determination of Kow does not characterize the partition of ionic surfactants (Tolls, 1998). On the other hand, it has been shown that the bioconcentration of anionic and non-ionic surfactants increases with increasing lipophilicity (Tolls, 1998). Tolls (1998) showed that for some surfactants, an estimated log Kow value using LOGKOW could represent the bioaccumulation potential; however, for other surfactants some 'correction' to the estimated log Kow value using the method of Roberts (1989) was required. These results illustrate that the quality of the relationship between log Kow estimates and bioconcentration depends on the class and specific type of surfactants involved. Therefore, the classification of the bioconcentration potential based on log Kow values should be used with caution.

A9.5.4 ***Conflicting data and lack of data***

A9.5.4.1 *Conflicting BCF data*

In situations where multiple BCF data are available for the same substance, the possibility of conflicting results might arise. In general, conflicting results for a substance, which has been tested several times with an appropriate bioconcentration test, should be interpreted by a "weight of evidence approach". This implies that if experimental determined BCF data, both ≥and < 500, have been obtained for a substance the data of the highest quality and with the best documentation should be used for determining the

bioconcentration potential of the substance. If differences still remain, if e.g. high-quality BCF values for different fish species are available, generally the highest valid value should be used as the basis for classification. When larger data sets (4 or more values) are available for the same species and life stage, the geometric mean of the BCF values may be used as the representative BCF value for that species.

A9.5.4.2 *Conflicting log Kow data*
The situations, where multiple log Kow data are available for the same substance, the possibility of conflicting results might arise. If log Kow data both ≥and < 4 have been obtained for a substance, then the data of the highest quality and the best documentation should be used for determining the bioconcentration potential of the substance. If differences still exist, generally the highest valid value should take precedence. In such situation, QSAR estimated log Kow could be used as a guidance.

A9.5.4.3 *Expert judgement*
If no experimental BCF or log Kow data or no predicted log Kow data are available, the potential for bioconcentration in the aquatic environment may be assessed by expert judgement. This may be based on a comparison of the structure of the molecule with the structure of other substances for which experimental bioconcentration or log Kow data or predicted Kow are available.

A9.5.5 ***Decision scheme***
A9.5.5.1 Based on the above discussions and conclusions, a decision scheme has been elaborated which may facilitate decisions as to whether or not a substance has the potential for bioconcentration in aquatic species.

A9.5.5.2 Experimentally derived BCF values of high quality are ultimately preferred for classification purposes. BCF values of low or uncertain quality should not be used for classification purposes if data on log Kow are available because they may give a false and too low BCF value, e.g. due to a too short exposure period in which steady-state conditions have not been reached. If no BCF is available for fish species, high quality data on the BCF for other species (e.g. mussels) may be used.

A9.5.5.3 For organic substances, experimentally derived high quality Kow values, or values which are evaluated in reviews and assigned as the "recommended values", are preferred. If no experimentally data of high quality are available validated Quantitative Structure Activity Relationships (QSARs) for log Kow may be used in the classification process. Such validated QSARs may be used without modification in relation to the classification criteria, if restricted to chemicals for which their applicability is well characterized. For substances like strong acids and bases, metal complexes, and surface-active substances a QSAR estimated value of Kow or an estimate based on individual *n*-octanol and water solubilities should be provided instead of an analytical determination of Kow.

A9.5.5.4 If data are available but not validated, expert judgement should be used.

A9.5.5.5 Whether or not a substance has a potential for bioconcentration in aquatic organisms could thus be decided in accordance with the following scheme:

Valid/high quality experimentally determined BCF value →YES:
→BCF ≥500: *The substance has a potential for bioconcentration*
→BCF < 500: *The substance does not have a potential for bioconcentration.*

Valid/high quality experimentally determined BCF value →NO
→Valid/high quality experimentally determined log Kow value →YES:
→log Kow ≥4: *The substance has a potential for bioconcentration*
→log Kow < 4: *The substance does not have a potential for bioconcentration.*

Valid/high quality experimentally determined BCF value →NO:
→Valid/high quality experimentally determined log Kow value →NO:

→Use of validated QSAR for estimating a log Kow value →YES:
→log Kow ≥ 4: *The substance has a potential for bioconcentration*
→log Kow < 4: *The substance does not have a potential for bioconcentration.*