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Fractionation and characterisation of AOX/EOX

A study of the present situation with respect to the physical, chemical and toxicological fractionation schemes for AOX/EOX

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SUMMARY

The AOX and EOX parameters are used to quantify the amount of organically bound halogens in complex environmental samples, for example in waste water. These values, however, are not directly correlated with the environmental effects of these organohalogens, since they measure a wide range of different substances, irrespective of their persistence, bioaccumulation or toxicity. The aim of this study was to search the literature for methods which are suitable for assessing the environmental impact of organohalogens in waste water. These might enable the impact to be expressed as an environmentally relevant sum parameter that could give a better indication of environmental impact than the concentration of AOX/EOX alone. Preferably these methods should be used in conjunction with fractionation schemes which would give additional information about any groups of environmentally hazardous organohalogen components present in the samples.

Although many different fractionation schemes are described in the literature, this report concentrates on the environmentally relevant ones. In particular, the report focuses on the following environmentally important fractions of AOX/EOX:

- The non-volatile fraction of AOX/EOX, which is defined as the group of substances with Henry's Law constants lower than 10 Pa m^3/mol.
- The bioavailable fraction of AOX/EOX, which is defined as the fraction after separating the fraction sorbed to the solid phase and that associated with macromolecular dissolved organic matter from the dissolved phase.
- The non-biodegradable fraction, for which it is possible to use the AOX value as a measure of the fraction of the organically-bound halogen mineralised.
- The potentially bioaccumulative fraction, which is related to the octanol-water partition coefficients of the substances present in the mixture.

Whilst these fractions do have relevance for the aquatic environment, these fractionation schemes can not be used directly to quantify the environmental risks of AOX/EOX. Furthermore, these published methods are often not validated and poorly characterised in terms of recoveries and they provide no additional information about the structures of the components in the different fractions. Equally, schemes which have been developed for the identification of individual components in certain types of AOX/EOX mixtures are also unsuitable for general use to assess the hazard of complex mixtures since complete identification of all the substances present is usually not possible and since the environmentally relevant properties of these substances are often unknown or are not readily available. Furthermore, such schemes are often specific for particular waste waters with their expected groups of organohalogens.

Despite these shortcomings, it is possible to propose integrated fractionation schemes which would give additional information about the comparative environmental impact of different organohalogen-containing mixtures. One such detailed scheme is proposed in this report (Section 4.1) but this would be too complex and time-consuming to be used routinely. It would also require further research to address a number of unknown factors and shortcoming. The most important of these are the overlap between the different fractions and the recoveries of the individual fractionations, such that a mass balance cannot be obtained for the scheme as a whole.

As an alternative, therefore, a simplified fractionation scheme designed for screening purposes is also proposed (Section 4.2). This scheme consists of steps which successively remove volatile, sorbed (non-bioavailable), biodegradable and non-bioaccumulating fractions from AOX/EOX mixtures, leaving the fraction which would contain any substances which are potentially persistent and bioaccumulating (the pPB fraction) and therefore, depending on their toxicity, potentially environmentally hazardous. As in the TEM (or WEER) method developed by RIZA to assess the environmental risk of complex effluents, it is proposed that these steps use standard techniques where possible.

The actual environmental hazard of this pPB-AOX/EOX fraction will depend both on the toxicity of the individual substances present and on the role played by other environmental fate processes not covered by the simplified scheme. Consequently, the pPB-AOX/EOX parameter would only be able to screen a range of different water samples to identify any which, bearing in mind likely quantities emitted, potentially contain significant quantities of persistent and bioaccumulating substances. Waste water containing significant pPB fraction needs further characterisation. This might involve additional testing of the abiotic degradability and anaerobic biodegradability, and some toxicity assessment, which may be achieved by tests for both acute and chronic effects and for mutagenicity. The difficulty of the toxicity tests here will be in separating the effects of halogenated compounds from those of non-halogenated ones also present in the pPB fraction. Alternatively, however, it may at this stage be possible to identify, or confirm the absence of, specific substances with known environmental effects or ecotoxicological parameters, since the pPB fraction may well be less complex than the original mixture. Despite the use of standard methods, however, the screening scheme may, at least at this stage, still be too complex for routine monitoring purposes.

To develop this simplified scheme for use in regulatory decision making, further work will be needed to test and validate it, using different waste water samples and mixtures of known organohalogens. In particular, the recoveries and mass balance of the individual steps should be determined, and the feasibility of identifying specific substances in the pPB fraction analytically should be explored.

Further development work would also be needed on methods to assess the anaerobic biodegradation and abiotic degradation of the pPB-AOX/EOX fraction, on methods for assessing acute and chronic toxicity and mutagenicity and on ways of differentiating the toxic effects of halogenated substances from those of non-halogenated ones.

SAMENVATTING

Het grote aantal organische halogeenverbindingen met uiteenlopende chemische en fysische eigenschappen, maar ook verschillend in potentiële milieubezwaarlijkheid maakt meting van deze verbindingen op component-niveau en beoordeling op milieubezwaarlijkheid in complexe milieumonsters erg moeilijk. Om organohalogenen te kunnen kwantificeren zijn de somparameters AOX en EOX geïntroduceerd. Er is echter geen direct verband tussen deze waarden en de milieubezwaarlijkheid van de monsters aangezien deze parameters een grote range aan organohalogenen met bepaalde gemeenschappelijk fysisch/chemisch eigenschappen kwantificeren zonder selectie op persistentie, bioaccumuleerbaarheid en toxiciteit. De onderhavige studie betreft een literatuurstudie naar bestaande mogelijkheden voor nadere karakterisering van de somparameters AOX/EOX op milieubezwaarlijkheid waarbij de milieubezwaarlijkheid als een somparameter uitgedrukt kan worden. Bij voorkeur zouden deze methoden gekoppeld moeten zijn aan fractioneringsschema's waarmee meer informatie verkregen zou kunnen worden over de groepen van milieubezwaarlijke organohalogeen-verbindingen in een monster.

Hoewel veel fractioneringsschema's voor AOX en EOX beschreven zijn in de literatuur, wordt in dit rapport de nadruk gelegd op schema's gebaseerd op milieurelevante eigenschappen. De volgende milieurelevante fracties van AOX en EOX worden in dit rapport beschreven:

- De niet-vluchtige fractie van AOX/EOX, welke bestaat uit de verbindingen met Henry-constantes onder 10 Pa m^3 /mol.
- De biobeschikbare fractie van AOX/EOX, welke is gedefinieerd als de overgebleven fractie na verwijdering van de fracties gesorbeerd aan de vaste fase en aan opgelost macromoleculaire organische materiaal.
- De niet-biodegradeerbaare fractie, waarvoor de AOX of EOX parameter gebruikt kan worden om de fractie mineraliseerbare organohalogeenverbindingen te bepalen.
- De bioaccumuleerbare fractie, welke is gerelateerd aan de octanol-water partitie-coefficiënten van de aanwezige verbindingen.

Hoewel deze fracties relevant zijn voor het aquatische milieu, zijn deze fractioneringsmethoden door het ontbreken van een directe realtie ongeschikt om de milieubezwaarlijkheid van AOX/EOX te kwantificeren als één somparameter. en hun milieubezwaarlijkheid. Bovendien zijn de fractioneringsmethoden niet gevalideerd en zijn de recovery's en de structuren van de componenten van deze fracties vaak slecht bekend. Fractioneringsschema's ontwikkeld voor de isolatie en identificatie van componenten in bepaalde soorten organohalogeenmengsels zijn eveneens ongeschikt voor algemeen gebruik omdat de identificatie van alle componenten niet mogelijk is en omdat de relevante eigenschappen van de verbindingen niet altijd bekend of beschikbaar zijn. Bovendien zijn zulke schema's vaak specifiek ontwikkeld voor bepaalde afvalwaterstromen met te verwachten groepen verbindingen.

Ondanks deze beperkingen kunnen geïntegreerde fractioneringsschema's wel gebruikt worden om meer informatie te geven over enkele milieu-gerelateerde kenmerken van AOX/EOX. Een dergelijk schema wordt in paragraaf 4.1 voorgesteld. Dit schema is echter te

complex en tijdrovend voor routinematig gebruik en heeft een aantal tekortkomingen waarnaar verder onderzoek nodig is. De meest belangrijke zijn de overlap tussen de verschillende fracties en de onbekende recovery's van de individuele stappen. Daardoor kan de massabalans van het hele schema niet bepaald worden.
Voor screeningsdoeleinden wordt in dit verslag een alternatief, vereenvoudigd schema voorgesteld. Dit schema bestaat uit stappen om de vluchtige, gesorbeerde (niet-biobeschikbare), afbreekbare en niet bioaccumuleerbare fracties te verwijderen. Hierdoor blijft de potentiële persistente, bioaccumuleerbare, en dientengevolge (afhankelijk van hun toxiciteit) de milieubezwaarlijke fractie over (de pPB fractie). Voorgesteld wordt om deze fractioneringsstappen te baseren op standaardmethoden zoals degene die gebruikt worden in de door het RIZA ontwikkelde TEM methode voor de bepaling van de milieubezwaarlijkheid van complexe effluenten.

De feitelijke milieu-effecten van de pPB-AOX/EOX fractie zijn mede afhankelijk van de toxiciteit van de verbindingen aanwezig in deze fractie en ook bijvoorbeeld van de rol van andere afbraakprocessen in het milieu. Hierdoor is deze parameter alleen geschikt voor het screenen van watermonsters op de aanwezigheid van significante hoeveelheden potentieel persistente en bioaccumuleerbare verbindingen. Indien dit het geval is, zou verdere karakterisering van de pPB-AOX/EOX fractie gericht moeten worden op het testen van abiotische en anaerobe afbreekbaarheid en op zowel acute en chronische toxiciteit (waaronder mutageniteit). Bij de toxiciteitstesten gaat gepaard met het probleem dat het niet mogelijk is te onderscheiden of de effecten afkomstig zijn van gehalogeneerde of van de niet-gehalogeneerde verbindingen . Verder zou het misschien mogelijk zijn de aan- of afwezigheid van specifieke verbindingen met bekende milieueffecten vast te stellen aangezien deze fractie minder complex van samenstelling zal zijn dan het oorspronkelijke mengsel. Ondanks het gebruik van standaardtoetsen, is dit screeningsschema waarschijnlijk te complex om gebruikt te worden voor routinematige monitoring.

Verdere ontwikkeling van dit screeningsschema zal vooral gericht moeten zijn op het testen en valideren ervan met verschillende afvalwatermonsters en met mengsels van bekende samenstelling van organohalogeenverbindingen. In het bijzonder zouden de recovery's en massabalans van de fractioneringsstappen en van het gehele schema bepaald moeten worden. Tevens zou de pPB fractie analytisch gekarakteriseerd moeten worden om specifieke verbindingen te identificeren.
Ook zou de aandacht bij de ontwikkeling gericht moeten zijn op methoden om anaerobe en abiotische afbreekbaarheid van de pPB-AOX/EOX fractie te testen; alsmede op methoden voor mutageniteit, acute en chronische toxiciteit en op de differentiatie van de effecten afkomstig van gehalogeneerde en niet-gehalogeneerde verbindingen in deze toxiciteitstesten.

1 INTRODUCTION

Around 7000 chemicals which are sold on the European market (about 10% of the total) consist of chlorine compounds. Nearly all of these are organochlorines (Goud et al., 1995), some of which are found on the grey or black list in many European countries.

The measurement of halogenated organics in water at the individual substance level as well as environmental risk assessment are complex matters due to the large number of organic halogen compounds with varying chemical en physical properties, and with varying potential environmental impact. This has given rise to the use of group parameters for monitoring purposes, such as the adsorbable organic halogens (AOX) and extractable organic halogens (EOX). These sum parameters quantify the amount of organically bound halogen on the basis of common physical and chemical properties. Part of the organohalogens, such as the polar organohalogens, are not included in these sum parameters. Another objection is that there is no direct relationship between the amount of the individual substances, for example expressed as AOX, and their environmental impact may vary strongly in different cases.

Like other group parameters such as BOD and COD, AOX/EOX plays a significant role in the reduction of organohalogen substances some of which could be harmful in the environment. The AOX/EOX parameter is mainly used as an indicator. In tackling diffuse sources, these group parameters have been used as a basis to prioritise products and other emission sources. Since there is no direct relationship between these parameters and environmental impact, this aspect has to be verified. On the whole, the AOX/EOX group parameter has been accepted internationally as a monitoring parameters for point source industrial effluent discharges. This can be an appropriate use where the proportions of different compounds in the discharge are stable, and their identity, or the environmental impact, is well characterised. In both cases, more research will be required to further characterize this group of halogenated organics in greater detail.

Growing concern to protect the marine ecosystem from terrestrial sources led to a (renewed) signing of The Convention for the Protection of the marine environment in the North East Atlantic in Paris in 1992. Its working structure on decision-taking level is named OSPAR. Its third level is made up of working groups, which are among others focused on the reduction of emission from diffuse (DIFF) and point (POINT) sources. At the meetings of the DIFF working group in 1995 and 1996 (DIFF, 1996), the deficiences of the AOX/EOX parameter were discussed. The need was stated for further investigations into more meaningful parameters than AOX/EOX and would be led by the Netherlands.

This report is the result of a literature study, which was commissioned by the Dutch Institute for Inland Water Management and Waste Water Treatment (RIZA). The aim of the literature study was to make an inventory of the present situation with respect of a further differentiaton of organohalogen mixtures by using physical or chemical fractionation and/or additional analytical techniques and its relationship to the AOX/EOX group parameter. This should lead to a method for a better assessment of the environmental impact of the waste water containing AOX/EOX. The literature study has been executed by the Department of Environmental and Toxicological Chemistry of the University of Amsterdam. The steering committee consisted of representatives from Lever B.V. (E.P. Beij), Procter & Gamble (V. Vandepitte, representing AISE), Solvay (C. de Rooij, representing the VNCI) and a consultant (J. Pickup), as well as RIZA (J. Plokker, G.B.J. Rijs).

This report consists of several chapters. After the introduction, the second chapter shows how the project was executed including certain limitations which could not be dealt

with completely because of time constraint. The third chapter gives an inventory of the present situation regarding fractionation for the chemical, toxicological and biological characterisation of the AOX/EOX parameter. In the fourth chapter integrated fractionation schemes are proposed which can be used to assess the environmental impact of the waste water containing AOX/EOX. The final chapter consists of the main conclusions and recommendations of this report.

2 EXECUTION OF THE PROJECT

The first part of the project consisted of a literature search in the Chemical Abstracts. This resulted in a number of scientific articles. Two searches were carried out: the first one led to a number of "hits" linked to the keywords AOX or EOX (818 hits) which could be reduced by introducing the keywords identif? or Anal? or Characteri? (223 hits) and by using the more recent literature after 1990 (160 hits). The abstracts contained a number of articles dealing with polymers, genetics and production. Omitting these, 110 hits were downloaded which gave around 30 useful articles. The second search used TIE, toxic?(W)identif? (119 hits) which was reduced to 74 by introducing only the more recent literature (after 1990). TIE is the aronym for toxicity identification evaluation.

Both searches led to cited articles which were judged on their titles and abstracts for further information. The most promising articles were screened for further references. This ultimately led to a number of articles dealing with the aim of the project in the open scientific literature. This usually consisted of references from scientific journals but very few articles or reports obtained from workshops or congresses because it takes time to obtain these. Some reports were requested and paid for but they have not been received yet during the period of the project. Apart from the scientific literature it was intended to obtain literature from the industry. No specific reports were received from the participating industries though two industry grpoupings, EUROCHLOR and AISE, commented extensively on the drafts and made suggestions for improvements to the proposed schemes. Thus the present report describes the state of the art in the official scientific literature regarding the aspects of identification and characterisation of the AOX/EOX sum parameter.

The second aspect was to establish written contact with the institutes active in the field of AOX/EOX sum parameters. These addresses were obtained from the references in the scientific literature and contacts with the participating parties and DIFF. The references were obtained by a direct or indirect link to the AOX/EOX sum parameter. Frequently, these articles dealt with experiments performed in effluents where paper/pulp bleaching was an important aspect.

The chosen literature was analysed in particular for the fractionation methods used in the characterisation of the AOX/EOX sum parameter: which methods were used, what was the result and to what extent was the objectives of the project met. Most effort was given to the recent literature. This has the advantage that the report is up to date but the disadvantage that it does not include much work on identification of the unknown substances and the environmental impact of substances which were a major concern around 10-20 years ago, such as dioxins and PCBs. In this report it is assumed further that the method to determine the AOX/EOX value is known to the reader. The main analytical characteristics in the fractionation methods are described in this report. For detailed information the reader is referred to the indicated literature.

3 FRACTIONATION SCHEMES FOR AOX/EOX

3.1 Introduction

The environmental impact of a mixture of substances can best be determined fully if *three aspects are known:*

1) which substances are present in the mixture and what are their environmentally important properties,
2) what are the concentrations of these substances and
3) *what effects do they have in each others presence (mixture effects).*

In general many substances may be present in a mixture in varying proportions. Group parameters have been developed to readily measure the total content of groups of substances in mixtures. The AOX and EOX parameters have been developed for mixtures of organohalogens. The AOX, in the past referred to as total organic halogen (TOX), differs from EOX in that in the first case one determines the total adsorbable organically bound halogen content of the mixture. The EOX value, on the contrary, depends very much on the solvent used in the extraction process.

Group parameters can also be applied as a starting point for the process of identification of individual components. The mixture is then fractionated and the different fractions may be measured in terms of the group parameter and/or characterised in terms of the chemicals present or in terms of other properties. Many fractionation schemes have been developed and it depends on the properties to be investigated which scheme(s) is (are) preferred. For the application of fractionation schemes, it is important to determine whether any artefacts occur. This can only be determined if a mass balance of the parameter (chemical) can be established. The use of mass balances in fractionation schemes is in general not very common. However, recoveries are frequently determined for single steps.

Sampling actual effluents can have the disadvantage that the content varies widely. Not only the concentrations of the substances present in the samples vary but the substances themselves may also be transformed as a result of different degradation mechanisms. Therefore, the AOX/EOX values of samples drawn at different times may change. However, it is possible that the relative sizes of different fractions of AOX/EOX remain the same although the absolute values may differ widely. A sequence of absolute values over a longer period may reveal certain important trends. Alternatively, it may be preferable to study model effluents which mimic particular processes or uses to eliminate some of this variation. Since degradation mechanisms may occur continuously in the sample, even after sampling, fractionation should start as quickly as possible. Either AOX or EOX could be taken as the starting point for the fractionation. However, due to the almost irreversible adsorption shown in AOX determinations, the absorbed fraction itself is not suitable for fractionation. In this case the AOX-containing water sample should be used, although this does introduce the possibility of nonhalogenated organic compounds also being adsorbed and interfering in some of the fractionation steps, such as those involving toxicity or extraction. Similar interferences are obviously possible for fractionations starting with EOX.

This discussion of fractionation starts with schemes which are used to obtain fractions defined in terms of environmentally important properties. These include both

physicochemical properties such as volatility and polarity as well as environmentally important properties such as persistence and bioaccumulation, although these properties are often related. In some cases fractions are operationally defined and are therefore surrounded by other problems. The schemes described usually deal with specific aspects of the AOX/EOX substances. During the discussion of the schemes some attention is given to the type of compounds which may be present in the fractions and their potential environmental impact.

Ultimately, the sum of the fractionated AOX/EOX values should give a total AOX/EOX value more or less similar to the one determined at the beginning of the procedure. The use of these AOX/EOX values as a kind of mass balance in these schemes has not been described in the literature. One of the main problems in achieving a mass balanced AOX/EOX fractionation scheme is the problem of losses during the process. This is especially important for two reasons: 1) liquid-liquid extractions and fractionations may have different recoveries for different components and may lead to significant losses, for example to the air and 2) (bio)degradation may result in the formation of degradation products with different recoveries. Extraction efficiencies of different organohalogens are indeed quite different. For example, the extraction efficiencies of a range of organochlorines using petroleum ether have been determined to be in the order of 80%, for a group of brominated ones they were around 60% and for iodinated compounds around 40%. The fluorinated ones were not extracted at all with petroleum ether (Wegman and Greve, 1977).

3.2 Comparison of extraction methods for AOX/EOX

Liquid-liquid (LLE) or solid phase (SPE) extractions play an essential role in AOX and EOX determinations. An important aspect in both adsorption and extraction processes is the recovery of the substances. AOX recovery data are remarkably dependent on the adsorbent used. The AOX data are almost entirely used as a comparison between different samples. The AOX values are more meaningful if it is known how comparable other adsorption methods are compared to these values. Comparison of the adsorption capacity of GAC and XAD-4 and extraction recoveries was made by Martinsen et al. (1988) and showed no difference for a single substance used (2,4,6-trichlorophenol) but was significantly different for a mixture (spent bleach liquor). A dilution of a factor of 60 reversed the adsorption capacities of GAC and XAD-4. An investigation of the extraction efficiency of a combined apolar/polar and apolar extraction solvent from an organic matrix (perch fillet) or sediment did not give statistically different EOCl values in the case of the organic matrix but it did with the sediment sample. Extraction of spiked sediment by cyclohexane only showed different results. Tetrachlorocatechol and 3,4,5-tricatechol were recovered very poorly (1%) which was explained by the authors on the basis of an oxidation process during the experiment or of irreversible binding to the sediment. However 4,5,6-trichloroguaiacol was extracted better than the tetrachlorinated congener because of its lower hydrophobicity. Investigation of AOX and EOX by thin layer chromatography (TLC) showed that the amount of lipophilic organohalogens (log K_{ow}>3) in a spent bleaching liquor sample is ~ 0.1% of AOX and ~10% of EOX while the distribution of molecular weight (by GPC) indicated that ~90% of the effluent had a MW <300, which was ~30% in fish and ~40% in sediment. Extraction recoveries varied but the may be caused by the influence of DOM (dissolved organic matter) in the sediment, which has not been accounted for.

AOX

Adsorption to XAD and Tenax is followed by a desorption step and the amount of halogen is then determined. For most organic halogens recoveries are high even for the volatile, polar and apolar compounds (Grøn 1990). Data were compared with those for other experiments with XAD, but from the reference it was not clear whether the differences were significant. Recoveries of purging were also given and it was clear that they decreased with decreasing Henry's Law Constants. The recoveries of chloroalkanes were high (>75%), average for chlorobenzenes (50-65%, although it was not clear whether a difference was found between each chlorobenzene) and negligible for chlorophenols and more hydrophobic organohalogens like aldrin, lindane and PCBs.

Laniewski et al. (1988) compared XAD-8 adsorption to that using GAC. It seems that XAD-8 does not adsorb as much of the substances present in precipitation as GAC does. Stripping enrichment of organohalogens in rainwater showed only 1,4-dichlorobenzene. Some organobromine peaks were found but were under the identification limit. The major contribution of low molecular weight substances (M<1000 D) to AOX in rainwater was found to be caused by neutral, non-volatile compounds. Of these, trichloroacetic acid was the most important one, but only accounted for around 10% of the measured AOX.

There is a major disadvantage in using GAC to obtain the starting sample for the fractionation of AOX. The adsorption is extremely strong and effective. Many substances are irreversibly bound, which means that desorption and subsequent determination is not possible. Furthermore, the carbon surface is an active surface, in that it most likely promotes radical reactions able to dimerise phenols (Chin et al.,1989). GAC is therefore not suitable for the identification and characterisation of AOX substances. In the literature other solid phases are sometimes used. However, the use of new examples of graphitized carbon black (GCB) has been investigated recently (Crescenzi et al., 1996). These have the advantage that they possess anion-exchange adsorption sites on their surface. Traditionally, extraction methods were used to concentrate the dissolved substances in the different water types. Recently other convenient solid-phase extraction techniques have been developed as alternatives to extraction using solvents. Of these, C18 bonded to silica has become especially popular. However its adsorption power for polar substances is limited. For these compounds other adsorbent materials being investigated such as cross-linked styrene-divinylbenzene copolymers or graphitized carbon black. The adsorption and desorption power of these materials was investigated in humus rich water spiked with a number of (very) polar and partially ionic pesticides. In the case of GCB no pH adjustment of the aqueous sample was necessary because it contains anion-adsorption sites. Carbograph 4 (C4) and 5 in particular were able to adsorb very polar substances whereby C5 displayed a greater amount of irreversible adsorption sites. Recoveries for the C4 and C5 were in general higher than 90%, especially if four times more carbon material was used. Different back elution schemes were used. An acidic or basic eluent could effectively desorb the acidic and basic organic substances.

EOX

The extraction of organic substances in EOX determinations depends strongly on the type of solvent used. Reemtsma and Jekel (1996) investigated the extraction effect of three solvents, methyl-tert.butyl ether, toluene and ethyl acetate, for determining the EOX of contaminated soil, lake sediments and sewage sludge. Soxhlet extraction with ethyl acetate proved most efficient and yielded 2-6 times the EOX values obtained using hexane. Interference by coextracted inorganic halides was negligible if dried samples were extracted.

This was even so for strongly hydrophobic substances: a spiked sample of PCB was extracted better by ethyl acetate than hexane from three sewage farm soils.

Schwantes and McDonough (1994) developed a fractionation scheme on the basis of ether extraction and the fractions were characterised in terms of relative size and chlorine-to-carbon ratio. Using ether implies extraction of low molecular weight and mildly polar organic substances. Ether extraction was performed for 96 h (one fraction) followed by a 336 h extraction. The 96 h extraction was followed by an acid, neutral and basic (phenolics) extraction. However the acid extraction was done with $NaHCO_3$ which is weakly basic in water.

The use of different solvents to define certain fractions has been described in the literature by Hendriks et al. (1994). These extracted fractions correspond more or less to different polarities or octanol-water partition coefficients of the organohalogens. Some liquid-liquid extraction (LLE) methods used to obtain chemically defined fractions are described in Table 1.

TABLE 1 MAIN CHEMICAL CLASSES OF EOX IDENTIFIED AFTER EXTRACTION UNDER DIFFERENT CONDITIONS

Ph	Sample	Extraction solvent	Analysis	Chemical classes	Reference
12	Sediment	Ether	GC-MS-SIM	Chlorophenolics Resin acids	Tavendale et al 1996
2	BKME	Diethyl ether	GC-MS-EI	Chloroacetic acids (85%) Chlorocarboxylic acids Chloro-oxo-pentenoic acids	Lindstrom et al., 1986
1	Well water	CH_2Cl_2	GC-MS-SIM	Atrazine + degr. Prod.	Gron, 1995
2	BKME	None	UV	Total + conjugated phenols	Verta et al. 1996
2	BKME	Petr.ether/acetone/ methanol	GC-FID	Fatty + resin acids	Verta et al. 1996
2	BKME	CH_2Cl_2	GC-MS	Sterols	Verta et al. 1996
12 and 2	Waste water	CH_2Cl_2	GC-MS-EI and LC-MS-DCI	Carboxylic acids + alcohols/surfactants + aldehydes/ketones+ esters+ halogenated aromatics+ hydrocarbons/ethers+ N+S-nonhetrocyclic + phenolics + plasticers + PAHs + steroids	Clark et al. 1991
1	BKME + Cl_2	Ether	GC-MS and GC-FID	Small halogenated saturated and unsaturated carbon compounds, C = 1-4	Kringstad et al. 1981

Clearly, the adsorbent or extracting solvent has an important influence on the compounds isolated by these methods. However, there is no direct relationships between these different fractions and their environmental impact, although in general the less polar fractions will contain potentially bioaccumulating compounds. Since it is at this stage unwise to neglect the environmental impact of any particular fraction a comprehensive fractionation scheme should include extraction and/or adsorption steps using a range of solvents or extractants in order to capture the widest possible range of AOX/EOX components. The environmental impact of these fractions should then be investigated.

3.3 *AOX/EOX and volatility*

Many fractionation and identification schemes described in the literature start with purging, leading to the fraction of purgable or volatile organohalogens. The criteria for a substance to be purgable is the Henry Law's Coefficient (HLC, Pa m^3/mol). To a first approximation this value can be calculated as the ratio of the vapour pressure and the (aqueous) solubility. The value HLC depends strongly on the temperature. In general, substances with a HLC higher than 100 are quite volatile while those with values between 10 and 100 can be still be purged successfully. Purging can take place in two ways: with nitrogen or with air. Although the use of air is convenient, it contains oxygen which may oxidise part of the volatile organic halides. Purging with nitrogen is preferable since it leaves a minimally disturbed non-volatile fraction. Purging with nitrogen and adsorption onto granulated active carbon (GAC) gives the purgable organic halogens frequently referred to as volatile organic halogens (VOX), which can also be termed AOX-volatile. The efficiency of adsorption of volatile halides onto GAC is, however, not constant. In particular dichloromethane and vinyl chloride do not seem to be adsorbed effectively (Grøn, 1990). For analytical purposes a different adsorption material is usually used (e.g. XAD) since desorption is easier (and thus the adsorption capacity is less), although successful desorption from GAC using carbon disulphide has been reported a few times (Biziuk and Przyjazny, 1996).

Volatile AOX/EOX appears to be only a small fraction of the total volatile organic fraction. For example, pentane extraction of unpolluted Swedish waters, followed by GC-ECD analysis showed that no volatile organochlorine compounds were present at above 1 ng/l in humus-rich water (Grimvall et al., 1994), whereas high concentrations of volatile non-halogenated compounds were found. Volatile organohalogens such as trichloromethane, 1,1,1-trichloroethane and tetrachloromethane accounted for only 0.1% of the total AOX concentration.

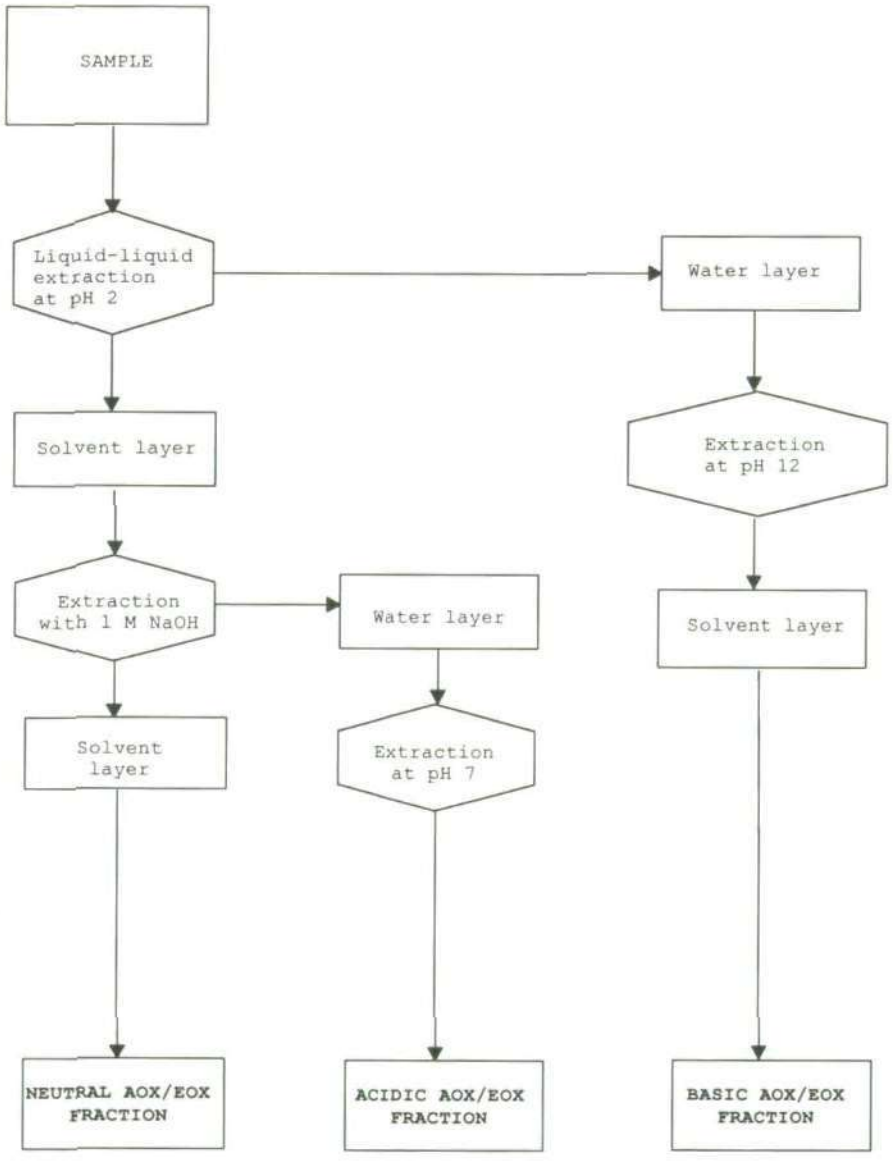
The methods used to determine VOX are well developed and are routinely applied. In the environment, the volatile organohalogens are considered to rapidly escape from the water phase and therefore do not lead to harmful effects on aquatic organisms.

3.4 *AOX/EOX and pH*

In many cases, particularly those concerned with pulp-bleaching effluents, fractionation schemes are based on variation of pH followed by either extraction or adsorption (see, for example, Scheme 1) leading, in general, to three fractions; an acidic (proton donating) one at around pH 2, a neutral one and a basic (proton accepting) one at around pH 12. These distinctions are caused by the pK_a and pK_b values of the substances present. In most cases the first two fractions are considered in terms of environmental impact. This is because the first (acidic) fraction contains the phenolic substances frequently causing the (toxic) effects found in the original samples. Apart from the phenolic substances, fatty, resin and other organic acids are found in this fraction. The neutral fraction contains neutral organic compounds. The basic fraction has received relatively little attention, but based on their pK_b values, this fraction should contain nitrogen compounds such as some pesticides. An AOX or EOX value can be determined for each fraction.

The extent of adsorption of AOX depends heavily on the pH. In general the AOX quantified increases with lower pH. This is caused by the protonation of ionic carboxylate groups in the humic structures (Laniewski et al., 1993).

Lindstrøm and Osterberg (1986) characterised and identified a number of acidic low molecular weight compounds in a BKME (bleach kraft mill effluent) effluent using ultrafiltration (<1000 Da fraction) and ether extraction and determined the organically bound chlorine in the various fractions. Identification was performed with GC-MS after isolation and methylation of the acidic components. A total of 31 acids were identified and quantified, most of them chlorinated. The low molecular fraction contained 25% of the total organically bound chlorine, of which 12% was extractable with ether. Of this 12%, 7% was acidic in nature, 4% phenolic and 1% neutral. No information was given on the identity or environmental effects of these compounds. In many cases no data was available on their toxicity. Most of these compounds occur as salts and may not be easily adsorbed or bioaccumulated.



Scheme 1 An example of a fractionation scheme based on liquid-liquid extraction at different pH.

Clark et al. (1991) set up an analytical system to identify non-regulated pollutants in some treatment plants. The concentration step consisted of a liquid-liquid extraction of the sample with dichloromethane followed by sequential extractions at pH 12 and pH 2. A second concentration step was carried out with XAD-2 followed by elution with dichloromethane and methanol. Both extraction methods only yield the low molecular weight (LMW) fraction. GC-MS-EI-DS and LC-MS-DCI-DS were used as detection instruments. With the first detection system 322 different compounds were identified in each extract of chlorinated wastewater effluents from three facilities. A total of 440 identified compounds was present in all the samples. Twenty-four halogenated compounds were identified in the three samples of which only a chlorotoluene isomer was found in each effluent. Of the 322 identified compounds, 181 were considered to be of commercial origin although it was not stated whether this included possible degradation products. Of these data only 49 were known at that time to possess carcinogenic and/or genotoxic activity. Of these 49 only 7 were chlorinated. No quantification was attempted.

Although fractionation of AOX/EOX and other pollutants on the basis of pH has been applied many times, it has been directed towards the identification of pollutants. There is no relationship between the acidity of organohalogens and their toxicity. The lack of data on the environmental effects of many of the compounds identified prevents the direct assessment of the environmental impact of the different fractions.

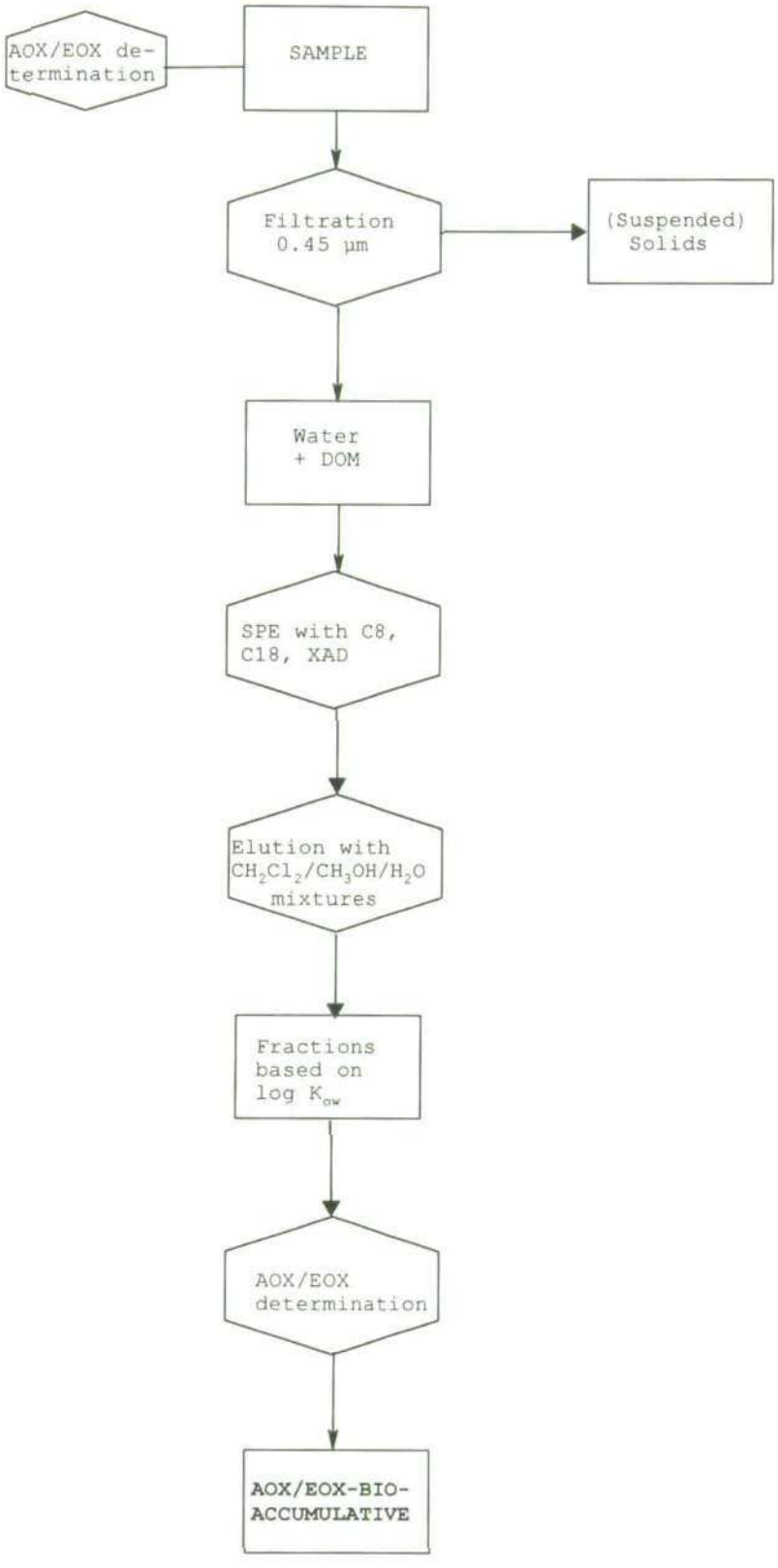
3.5 AOX/EOX and bioaccumulation

The accumulation of organic compounds by organisms, or bioaccumulation, can lead to undesirable effects of these compounds, not only on the accumulating organism but also by transfer through the food chain to organisms in higher trophic levels. Bioaccumulation from water is called bioconcentration and the relationship between the log BCF (bioconcentration factor) and log K_{ow} is in general linear in the log K_{ow} range from 2 to 5. Above log K_{ow} 5 the log BCF no longer increases and indeed sometimes decreases (Loonen, 1994). In general, the fraction responsible for bioaccumulation contains compounds with log $K_{ow} > 3$.

The AOX/EOX fraction with a potential for bioaccumulation has been described in the literature by Durhan et al. (1993), based on the log K_{ow} values. They described a fractionation scheme suitable for fractions from log K_{ow} 2.5 to 7. Scheme 2 shows the main components of this scheme. Filtration of the effluent took place through a 0.45 µm filter and as such the filtrate was defined as the soluble phase (including DOM). Elution of the high log K_{ow} (2.5 to 5) fractions was carried out using solid phase extraction followed by elution with varying ratios of water, methanol and dichloromethane. The latter is a very volatile liquid which can easily be removed by heating the solution for some time at 40°C. This is necessary if the fractions are to be tested for toxicity as dichlormethane has a high toxicity. Good recoveries were obtained for a test mixture of PAHs and other hydrophobic compounds. This test mixture had log K_{ow} values calculated with the CLOGP method. This methods overestimates the high log K_{ow} values so that the true values might be (slightly) different. Using this method, eleven bioaccumulative compounds were identified in sediment pore water, but not named in the publication. No further characterisation of their environmental impact was carried out.

An alternative method is to fractionate on the basis of octanol-water partition coefficient or bioconcentration factor using reversed phase HPLC, as has been applied by the

Dutch National Institute for Coastal and Marine Management (RIKZ) to mixtures of unknown compounds (Klamer and Beekman, 1995).



Scheme 2 Tentative scheme to obtain the AOX/EOX-bioaccumulative fraction based on Durhan et al (1993).

Hynning (1996) developed a procedure for the separation, identification and quantification of bioconcentrating components of industrial effluents. The effluent was first extracted with a mixture of hexane/t-butyl methyl ether. Then, the extracts were separated by a semi-preparative HPLC with a gradient elution using a C18 column and a mobile phase of phosphate buffer and methanol. Reference compounds in the log K_{ow} range from 1.4 to 4.7 recommended by the OECD were used to identify three fractions: a log K_{ow}<3, a log K_{ow} 3-5 and a log K_{ow}>5. Components of these fractions were derivatized and chromatographed on a column of silica gel before identification with GC-MS. The silica gel columns were eluted with different solvents: hexane for neutral compounds such as hydrocarbons, benzene for carboxylic acids esters and phenol acetates, and hexane/t-butyl methyl ether for the more polar compounds. Quantification was made after identification with GC-FID with suitable internal standards. Sixteen compounds with log K_{ow} >3 were identified and quantified in a sample of a pulp mill effluent before and after physical chemical treatment, although this represented only 7% of the total extract. 2,4,6-Trichlorophenol, 3,4,5-trichlorophenol, 14-chlorodehydroabietic acid and 12,14-dichlorodehydroabietic acid were among the chlorine compounds identified but in low quantities. The other compounds were high carbon saturated and unsaturated fatty acids (>C12). In one sample of a mill, handling recycled paper, identified compounds with log K_{ow}>3 consisted only of saturated and unsaturated high carbon fatty acids. Fractionation on molecular weights was not included in the set up, but may be a valuable complement.

Recently, another form of solid phase extraction has been used to quantify bioaccumulative compounds in water samples (Verhaar et al., 1995, Van Loon et al., 1996). In this case C18 Empore disks were used to extract hydrophobic compounds from water, which were quantified as a total molar concentration by means of vapour pressure osmometry and extrapolated to the total body residue. Possibly, this interesting technique could be coupled to further fractionation and organohalogen determination. A further new technique is solid phase microextraction (SPME) which is a miniaturised form of SPE and has been used to determine the freely dissolved concentration of hydrophobic compounds (Vaes et al., 1996).

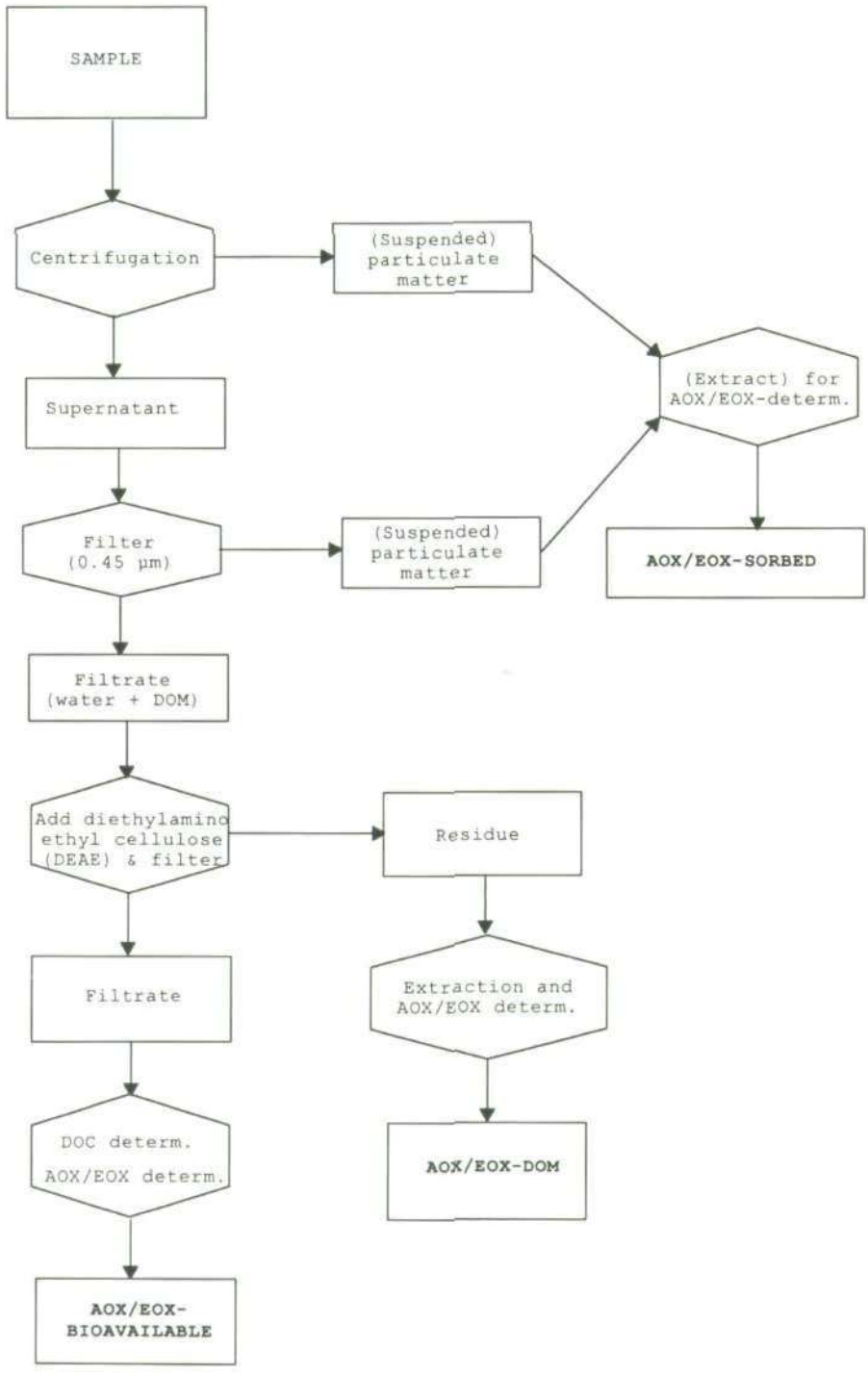
Estimating bioaccumulation from log K_{ow}, such as is the case here, does ignore the possibility of the organohalogens being metabolised in the organisms. As the metabolites of hydrophobic organic compounds are generally more polar than the substrates, this could mean that the actual bioaccumulation of organohalogens would be less than that estimated from log K_{ow}.

The difficulty of identifying bioaccumulative compounds and the lack of information on their toxicity makes it difficult to assess their environmental impact. However, it is possible to estimate the baseline toxicity of fractions of known log K_{ow}, since there is a good correlation between the baseline (narcotic) toxicity of certain organic compounds and their log K_{ow} (or BCF) (Verhaar et al., 1995).

3.6 AOX/EOX and bioavailability

Although the presence of hydrophobic, and therefore potentially bioaccumulative, organohalogens can be determined by methods such as those described in the previous section, in reality, bioaccumulation will compete with other distribution processes. Sorption to particulate and dissolved natural organic matter is particularly important for hydrophobic

organic compounds and can reduce the bioavailability, and thus bioaccumulation, of potentially bioaccumulative organohalogens. This is because the uptake of compounds by aquatic organisms is considered to take place from the freely dissolved phase. This phase will contain predominantly more polar organohalogens.



Scheme 3 Global fractionation scheme to obtain bioavailable, DOM or third phase and sorbed fractions of AOX/EOX.

Scheme 3 shows a fractionation scheme in which different more or less operationally defined fractions which can be related to bioavailability are created. This scheme is based on

the one used by Burnison et al. (1996) although some slight modifications have been introduced. The AOX/EOX value of the original sample can be determined and this can be regarded as a total AOX/EOX value. The AOX/EOX values of the different fractions may be used to determine the AOX/EOX mass balance.

In the first step the sample is centrifuged and filtered. Filtration is a frequently applied using 0.45 μm filters. The filtrate contains the third phase or dissolved organic matter (DOM, including sorbed organohalogens) and the "freely dissolved" organohalogens. The residues after centrifugation and filtration are defined as the solid phase. The particulate matter can be extracted to obtain the sorbed EOX value. The filtrate can be used for solid phase extraction (SPE) or liquid-liquid extraction (LLE) procedures. Since the adsorption capacity of the used solid or liquid phase is in general lower that of GAC, an AOX/EOX determination of the filtrate after extraction should be done in order to obtain a mass balance. The separation of the third phase from the filtrate is much more difficult. In fact, it is generally believed that complete separation is impossible. There are some methods available to separate the DOM from the solution but it is not clear at all how successful they are. For example, Burnison et al. (1996) used diethylaminoethyl cellulose (DEAE) to adsorb the third phase but it is not clear how effective this process is. This method is included in Scheme 3. A newly developed alternative method is solid phase microextraction which has been successfully applied to the analysis of dissolved fractions of organic compounds (Vaes et al., 1996). Another way to separate the DOM phase from the water is using RP-HPLC (Landrum et al, 1984). However, it seems that complete separation is still not achieved. The freely dissolved organohalogens are generally regarded as being the bioavailable, and therefore the potentially environmentally harmful, fraction and may be used for identification purposes and further characterisation.

The DOM or third phase fraction is potentially important for highly hydrophobic substances. Depending on the concentration of DOM, the concentration of freely dissolved hydrophobic substances may be extremely low and most of the compounds may in fact be sorbed to DOM. There is ample evidence indicating the fraction sorbed to DOM is not directly bioavailable, but this is potentially bioavailable.

The exact composition of DOM is the subject of much investigation. One idea is that large DOM molecules contain hydrophobic fragments or that DOM molecules form micelles suitable for the sorption of highly hydrophobic (organohalogen) compounds present in the solution. Separation on the basis of molecular size, or more commonly on molecular weight, using size exclusion chromatography (SEC) is an established method, whereby the fraction above 1000 Da is generally regarded as non bioavailable. However Jokela and Salkinoja-Salonen (1992) showed that a dilution factor of 100 changed the weight fraction pattern to lower sizes, indicating that DOM shows a micellular type of behaviour. It is possible, however, that hydrolysis may occur but the rate of hydrolysis of ester bonds is in general too low to cause this effect.

It should be borne in mind that AOX/EOX measurements do not distinguish between low molecular weight organochlorines sorbed to DOM and chlorinated DOM molecules. In the first case, the AOX/EOX is potentially bioavailable whereas in the second case this is not so. A separation method to distinguish between these two different fractions should therefore be developed. In addition, a method to measure the potential bioavailability of sorbed AOX/EOX is also required to completely characterise the bioavailability of AOX/EOX as a whole.

The bioavailable fraction is thus defined as the fraction containing the freely dissolved substances. However, the fact that the adsorbed fraction is potentially bioavailable is ignored

in such a scheme. Each individual component of AOX/EOX will be distributed between dissolved (i.e. bioavailable) and sorbed (nonavailable) phases. This distribution process may be in an equilibrium which will depend on the physical chemical properties of the compounds, in particular their hydrophobicity, as well as environmental factors. As soon as the sorption equilibrium is disturbed, for example by uptake of the dissolved fraction by organisms, sorbed compounds may be released and thus become directly bioavailable. The importance of this process depends on the rates at which the adsorbed fraction is released. Therefore, the division of AOX/EOX, as well as other compounds, into bioavailable and non-bioavailable fractions is not absolute. Furthermore, no account is taken of the direct uptake of sorbed organohalogens in the particulate phase, for example by filter feeding organisms or those feeding on sediment particles.

3.7 AOX/EOX and (bio)degradation

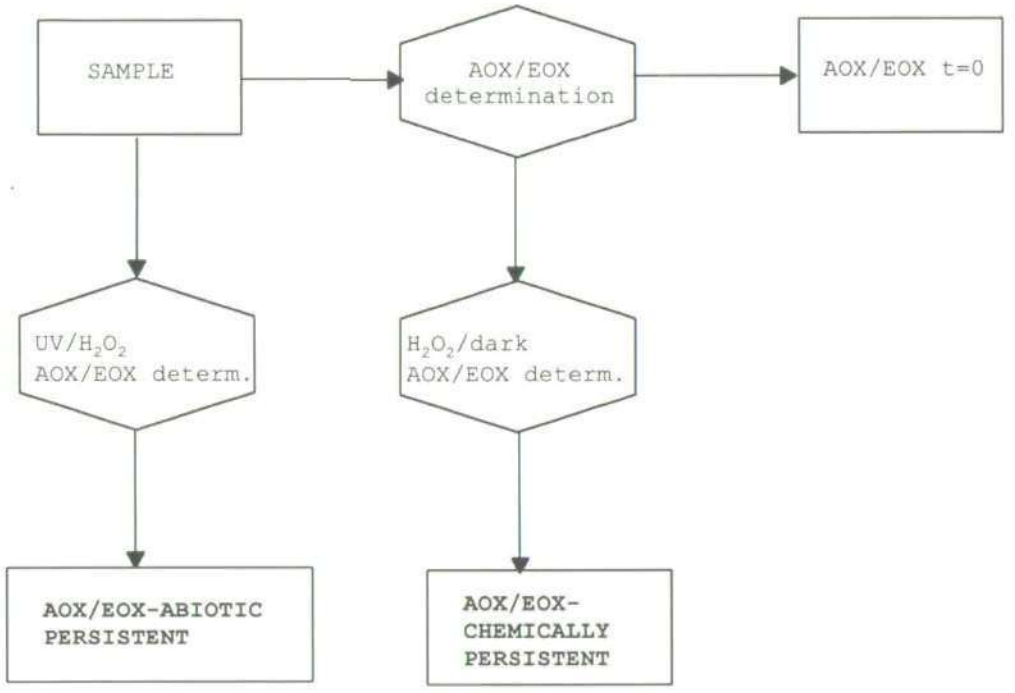
Each substance released into the environment is subjected to degradation which, potentially, may continue until the substance is mineralised. Mineralization is defined here as complete degradation of the substance to carbon dioxide, methane, water and chloride ions. The halogens which play the most important role in the AOX/EOX determination are released as halide during mineralization of the organohalogens. This means that an AOX/EOX determination has a great advantage in that it might be used as an indicator for removal of organically bound halogens by measuring the decrease in AOX/EOX.

Degradation can be divided into abiotic and biotic processes. If abiotic degradation is to be investigated the sample should not contain any active degrading organisms. Hydrolysis, oxidation, reduction and photolysis are the main abiotic degradation mechanisms. Abiotic degradation of substances is frequently investigated in the laboratory with UV light and hydrogen peroxide as a model for water purification. Under the influence of UV/H_2O_2 the organohalogen will react as a reducing agent since H_2O_2 is a strong oxidising agent and produces in combination with UV light the highly reactive hydroxyl radicalsThis method could be adapted to the fractionation of AOX/EOX although conditions in the environment are generally milder.

Other reactions are possible under environmental conditions in which the halogen atom is released from the substance as an halide ion. This could be by an elimination reaction which may occur especially for the smaller chloroalkanes. Substitution by a hydroxide ion may also occur. A decrease of AOX/EOX in the dark is an indication for these processes, Alternatively, more environmentally realistic conditions could be used to determine the photolytic and chemical reactivity of AOX/EOX. At the moment there are no standardized test methods to study the abiotic degradability of chemicals. A scheme in which the photolytically or chemically persistent fractions of AOX/EOX could be quantified is shown in Scheme 4.

Abiotic degradation of AOX/EOX was investigated by Smeds et al. (1994), who measured under more extreme circumstances (high temperature, low and high pH and UV/H_2O_2). The chlorinated compounds were determined as AOX, EOX and as individual compounds using GC. EOX values were determined by acidic extraction with ethyl acetate which accounted for 5% of the AOX values. This percentage decreased after treatment of the effluent, which indicates that low molecular weight compounds should have a higher tendency to release organically bound chlorine under these extreme conditions. Increasing the pH and temperature of spent chlorination stage liquor showed that chlorophenols,

chloroguaiacols, chlorovanillins and chloroacetic acids were rather stable in both alkali extraction and C-stage liquor in the pH 2-12 range even at higher temperatures (100°C). Chlorocatechol concentrations decreased above pH 8, whereas chloroguaiacol concentrations increased under alkaline conditions and chlorovanillin concentrations increased in C-stage liquor above pH 4. Di- and trichloroacetic acids showed thermal degradation at high temperatures but at lower ones their concentration increases probably due to degradation of chlorolignins. Chloroacetones and some chlorohydoxyfuranones are unstable under neutral conditions, while the chlorinated butene-1,4 dioic acids were found to be quite stable. All treatments show an increase of dichloromaleic acid anhydride.



Scheme 4 A tentative scheme for the determination of abiotically persistent fractions of AOX/EOX.

The concept of mineralization of the organohalide is also be used in the case of biodegradation. Biodegradation is frequently thought of as a process leading to the disappearance of the contaminant. However, this primary biodegradation may lead to products which are even more toxic than the reactant(s). The main emphasis should therefore be on mineralization of the halogen component of the organic substances. Biodegradation of organohalogens can be an important environmental fate process and may be divided into aerobic and anaerobic processes. However, these terms can be quite misleading when dealing with organohalogens. In the case of aerobic biodegradation, organohalides are oxidised. The oxidising agent necessary is usually oxygen but nitrate or certain transition elements (iron or manganese for example) are also capable of oxidising organohalides under anoxic conditions. Similar reactions still occur as in the presence of oxygen leading to the incorporation of an oxygen atom into the organohalogen, initially leaving the C-Cl bonds intact.

True anaerobic biodegradation of organohalogens is a process in which the halides are liberated according to the reaction:

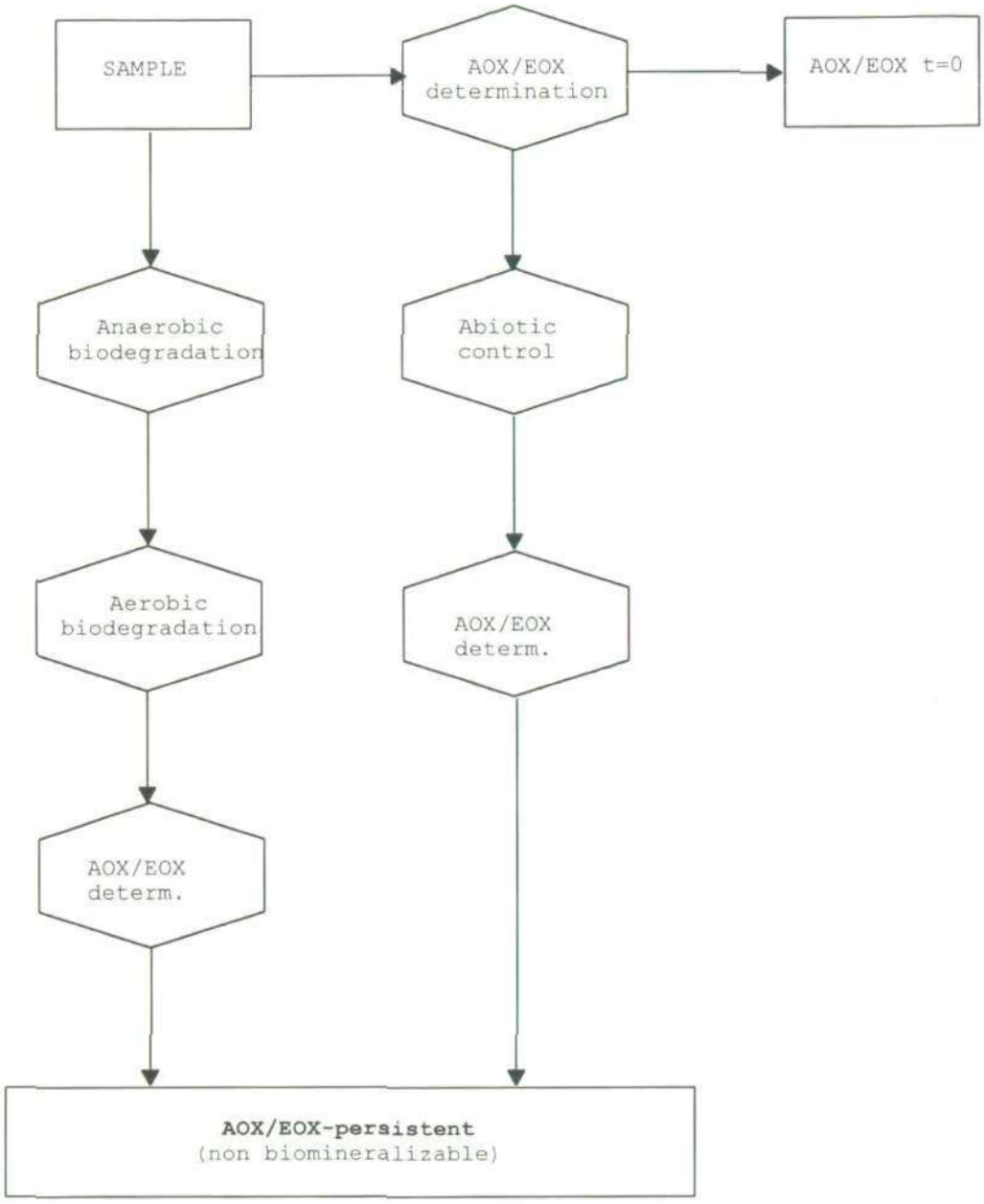
$$R\text{-}Cl + H^+ + 2\,e^- \rightarrow R\text{-}H + Cl^-$$

This process is called reductive dechlorination and it will only occur if the environment is substantially anaerobic, for example under sulphidogenic or methanogenic conditions. If reductive dechlorination occurs, the halide formed is removed during the AOX/EOX determination and the AOX/EOX value will then decrease. This may not be the case when the organohalogens are oxidised. Other organohalogens may be formed and will be measured again as AOX/EOX. A third type of reaction that may take place is hydrolytic dehalogenation, for example the substitution of a chlorine atom by a hydroxide group. Although not a redox reaction, this also reduces the AOX/EOX content.

Comparing both different types of degradation in terms of organohalogens, it may be concluded that oxidative degradation lead to products which are normally closer to complete mineralisation than reductive degradation, but that the latter process is more likely to remove halogens. Therefore, the C:Cl ratio increases when reductive dechlorination and substitution occurs but may remain more or less constant if the organohalogens are oxidised.

Standardised biodegradation tests, such as the OECD methods (OECD, 1993) are usually performed with activated sludge in an aerobic environment where organohalogens will be oxidised. Thermodynamic calculations show that this process is more difficult when more chlorine atoms are present in the molecule. On the contrary the more highly chlorinated the compound is, the easier it will undergo reductive dechlorination (Krop et al., 1994). From the point of view of AOX/EOX determinations, reductive dechlorination may lead to substantial reduction of the AOX/EOX value. However these processes are in general slower and it is not known to what extent these conditions will be encountered by the contaminant in the environment. Nevertheless, both processes should be included in a fractionation scheme such as that shown in Scheme 5. As far as possible, the standardised methods to test biodegradation should be used. Many of these tests use bacteria from sewage treatment plants (OECD, 1993) although methods have been developed in which sea- and freshwater water bacteria are used (OECD, 1993, Aquasense 1995). At the moment, there is no standardised test available for anaerobic biodegradation, although there is one in development (Nuck et al, 1996).

Biodegradation of AOX was followed under different circumstances related to the molecular weight distribution by Saski et al (1996). They found that anaerobic biotreatment was not inferior to aerobic in dehalogenating the chlorolignins and it seems that 50% of the total dehalogenation was already achieved prior to contact with biomass. It was found that only a part of the AOX compounds contribute to BOD. In anaerobic environments a slightly faster AOX degradation was found for the higher molecular size fraction while in aerobic environments it is the opposite. A minor fraction (10%) of the AOX was removed from the water column to the sediment during the year but the average molecular weight was around a factor of 10 higher with a maximum appearing around and over 1000 Da. A similar phenomenon was found in soils polluted with chlorophenols especially in the presence of humic acids. The authors propose extensive metabolism by the biota. Another reason, however, is more likely since hydroxyphenols may polymerise rapidly through a radical mechanism.



Scheme 5 AOX/EOX (bio)degradation scheme to obtain a persistent fraction.

The incorporation of tests for (bio)degradability into fractionation schemes for AOX/EOX is possible. However standardised methods will have to be developed in some cases, such as abiotic degradation and anaerobic biodegradation. Although biodegradation is generally regarded as reducing the environmental impact of pollutants, this is not always the case. It is possible that biodegradation or chemical degradation leads to the formation of products which are more toxic than the original compounds. Therefore the testing of the toxicity of any organohalogen degradation products of AOX/EOX should also be included in such a fractionation scheme.

3.8 AOX/EOX and toxicity

Although the relation between AOX/EOX and toxicity has been the subject of a number of investigations, no correlation could be established. This is because nonhalogenated compounds present in the samples may also contribute to the toxicity. Fractionation schemes have been developed to derive fractions containing toxic components. Of particular importance are the schemes developed by the US-EPA (Norberg-King et al., 1991, Lukasewycz and Durhan, 1992, Durhan et al., 1993, Mount and Norberg-King, 1993). Some important aspects in developing a fractionation scheme are: 1) toxicity tests should not be conducted for only one organism and 2) there should be a distinction between marine and freshwater organisms and 3) in the fractionation schemes special care should be given to the extraction solvents. They might be extremely toxic to the organism itself. A good choice and well defined blanks diminish the chance of artefacts.

The results of toxicity tests also depend on the organism chosen in the tests. Recently a battery of toxicity tests have been included in such studies and a number of group parameters have been determined in order to assess any possible correlation. One of the main problems is to interpret the results of the different toxicity tests. For example the environmental relevance of the Ames test for mutagenicity is still unknown while not all toxicity tests used may indicate a harmful effect. There are also differences in toxic aspects between freshwater and marine organisms.

Bullock et al. (1996) has investigated the influence of the high and low molecular weight fractions of a BKME on the microbial activity of activated sludge. The high molecular weight (HMW) component of the effluent tended to be resistant to biodegradation and has been shown to be the principal source of the AOX present in the discharged effluents. The fraction is in general hydrophilic and therefore does not bioaccumulate A separation was made between HMW and low molecular weight (LMW) fractions with a working definition of 1000 Da. Microbial activity was determined as biomass production and substrate removal (glucose). Most of the organic material (COD) was found in the LMW fraction but this fraction had a very low degree of chlorination. The microbial activity was diminished by both fractions. For the HMW fraction this was known before but for the LMW fraction this was not expected. Investigation of nutrient deficiencies showed that the LMW fraction was deficient in nitrogen. It was also found that no sorbed toxicant was released by the HMW fraction. Adding a nitrogen source increased the microbial activity substantially, but the relationship between the effects of toxicity and nutrient limitation was not clarified..

An extended toxicity test was set up by Verta et al. (1996). Toxic impacts in the past were mainly focused on chlorinated compounds but recent improvements in technology like ECF (elemental chlorine free) and TCF (total chlorine free) treatments have on one side reduced the concentrations of chlorinated organic compounds in these effluents to low levels, but have frequently not diminished the toxic effects. A battery of standard toxicity tests was used: tests with *Pseudomonas putida*, luminescent bacteria, *Daphnia* for acute toxicity and tests on the fertilised eggs and hatched larvae of the zebra fish for LOEC values and algal growth tests. Correlations were determined of toxicity with a number of chemical characteristics: BOD_7, COD_{Cr}, TOC, loss on ignition, suspended solids, colour, total nitrogen, total phosphorous and a serie of specific group compounds like dichloromethane extract, AOX, 25 major and trace elements, phenolic compounds (from the difference of UV adsorption at pH 12 compared to pH 6), fatty and resin acids (using petroleum ether/acetone/methanol extraction and GC-FID), molecular weight distribution (using gel chromatography), terpenes and sterols (using extraction with dichloromethane of the acidified

sample and GC-MS identification), EDTA and DTPA chelating power. In order to compare toxicity with effluent water quality, a toxicity index (TI) was defined on the basis of the most sensitive tests (luminescent bacteria (*V. fisheri*), algal growth (*S. capricornutum*) and eggs and larvae of zebra fish (*B. rerio*) : $TI = (TC_{vf} + TC_{Sc} + TC_{Brh} + TC_{Brg})/16$. Defined in this way the TI of the conventional BKME was only 30% higher than for an ECF and TCF effluent. Only after a secondary treatment did the TI value defined in this way decreased drastically. Correlation analysis was executed between the TI and the effluent chemistry. The toxic effluent was strongly related to the organic material. Of the specific compounds known to have toxic properties, phenols and fatty acids gave a fairly good correlation with toxicity.

The results could not be explained by the corresponding chlorinated compounds since AOX was hardly detectable in some of the toxic fractions. Stepwise multiple regression showed that COD was selected as explaining most of the toxicity variance and of the identified chemicals, 75% of the variance was explained by the phenols and the fatty acids. Although the resin acids concentration increased the explained variance slightly, its corresponding Fisher F-value was only 2.5 which is in general too low for a significant increase. However, in the original reference the resin acids are (wrongfully) included in the totally explained variance. The toxicity of pulp mill effluents seems to be a result of numerous bioactive substances rather than a specific compound. AOX cannot explain toxicity of pulp effluents at the current low AOX levels.

Craig et al. (1990) investigated the toxicity and bioaccumulation of AOX and EOX of BKME, river water, sediments and biota. EOX was defined after extraction by cyclohexane/isopropanol. Some individual compounds were also identified in the samples: 7 chlorinated phenols, 4 catechols, 5 guaiacols, 2 vanillins, 1 syringol and 10 chlorinated and non chlorinated resins, 9 fatty acids and 2 chlorinated sulphones. Standard identification methods for these substances were used. Acute toxicity tests with rainbow trout and chronic tests with fathead minnows and *Ceriodaphnia dubia* were carried out in the effluents. Sediment toxicity was determined by survival of the mayfly larvae and midge larvae. The presence of a toxic compound in sediment was also determined by eluting the sediment and testing the eluent with *Daphnia magna.*

The AOX/EOX concentrations in the different samples varied. Values were more or less in line with those expected for unaffected waters and affected waters based on available receiving water dilution. No relation was found between AOX and toxicity and bioaccumulation. Bioaccumulation factors of the identified substances were calculated. The authors claim that BAF values calculated by comparing body levels to water and food levels for DDT and PCBs were very high. The 24 chlorinated organics measured in the studies accounted for around 0.5% of the effluent AOX but represented 25-35% of the EOX from both mills. This was reduced to 10-19% of the EOX (chlorine based) in analysed sediment samples but was more than 75% of the EOX in the two different river benthos systems. All the compounds identified had molecular weight lower than 400. Based on these results and the results of other experiments with chlorinated phenolics, the authors concluded that the majority of chlorinated compounds contributing to the EOX in the effluent and sediment were either not available to the benthic organisms or alternatively were quickly excreted after ingestion. It was further concluded that monitoring of AOX and/or EOX in the environment does not appear to provide useful information with regard to biological protection or effect. It is more productive for environmental protection programs to measure, monitor and assess the effect of specific compounds that have been shown to be detrimental to the receiving environment.

A great deal of knowledge on the identification of AOX/EOX has been built up in the last 15 years at the KIWA-institute. They developed a fully automatic XAD isolation procedure for water samples. After gas stripping to measure the VOC, the water sample is extracted with XAD resins at several pH. The mutagenic effect in the XAD isolate at pH 12 is similar to the one at pH 7 while the XAD isolate at pH 0.1 seems to be higher in mutagenic character. The report (in Dutch) gives an extensive list of substances identified in different types of fresh water in the different fractions.(KIWA, 1996)

Toxicity testing of fractions of AOX/EOX has been applied extensively, in many cases using standardised toxicity test methods. The empasis has been on acute toxicity tests with less attention being paid to chronic test and tests for mutagenic effects. Tests for mutagenicity and other chronic effects should be included in methods for a comprehensive assessment of the potential environmental impact of AOX/EOX.

3.9 Unknown factors in the AOX/EOX fractionation schemes

AOX/EOX can be fractionated, in many cases using well-known methods, into crudely defined fractions which can be used to make simple assessments of the environmental impact of AOX/EOX. However, a responsible assessment is only possible if the composition is known and the environmentally relevant properties of the components are also known. Although in some cases there is information available on the composition of AOX/EOX, information on the environmental behaviour and effects of these components is incomplete. Therefore, there is a place for using defined fractions of AOX/EOX to assess the environmental impact of these mixtures. However, there are a few areas where more information is required.

1. Fractionation schemes are based on equilibrium considerations. In reality, non-equilibrium process such as desorption contribute to environmental impact. More information is required on the kinetics of such processes in order to assess the environmental impact of AOX/EOX.
2. As an example of a nonequilibrium process as mentioned above, methods are required to assess the potential bioavailability of sorbed AOX/EOX.
3. The relationship between low molecular weight AOX/EOX sorbed to dissolved organic macromolecules and high molecular weight AOX/EOX should be investigated.
4. Standardised methods to test anaerobic/anoxic biodegradation and photolytic and chemical degradation in environmentally realistic ways are needed.
5. Ideally, a method to distinguish the toxic effects of AOX/EOX from those of nonhalogenated compounds also present in environmental samples is required, although this is likely to be difficult to achieve.
6. More effort should be devoted to achieving complete mass balances in fractionation schemes.

4 PROPOSED INTEGRATED FRACTIONATION SCHEMES

The fractionation methods mentioned and discussed in chapter 3 and the properties on which they are based are shown in Table 2.

TABLE 2 MAJOR FRACTIONATION METHODS APPLIED TO AOX/EOX AND THE PHYSICAL CHEMICAL PARAMETER THEY ARE BASED ON

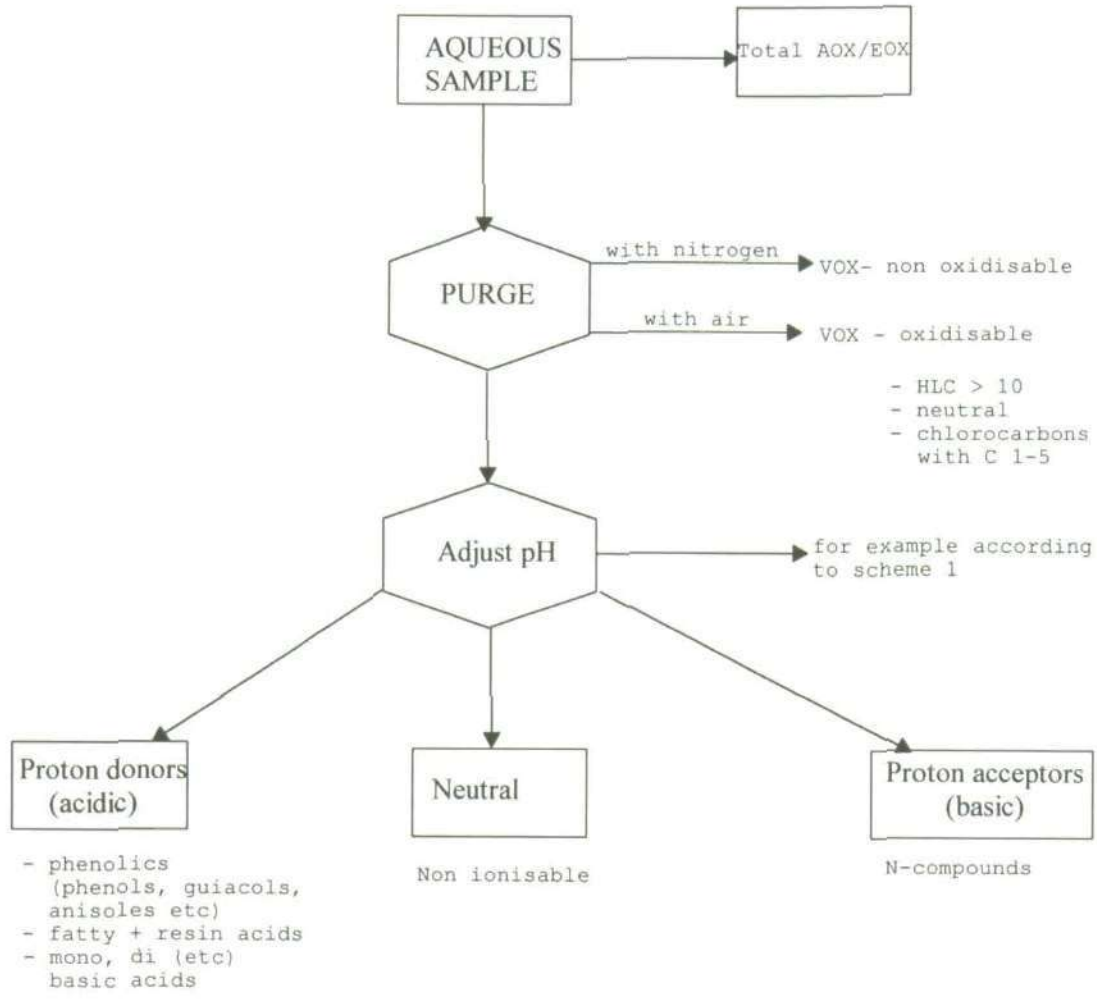
Method	Parameter
PHYSICAL METHODS	
pH variation	pK_a or pK_b
Solvent extraction	Polarity
Residue after 0.45 µm filtration	(contaminated) particulate
Extraction/SPE of filtrate after 0.45 µm filtration	*(contaminated) DOM/third phase*
Filtrate after removing solids and third phase	Freely dissolved
Size exclusion chromatography/ultrafiltration	Molecular size/weight
Purging with nitrogen	Volatility (HLC >10)
HPLC methods	log K_{ow} and/or chemical class separation
CHEMICAL METHODS	
Purging with air	Volatile oxidative
Standard aerobic biodegradability tests	Oxic biomineralizable
Anaerobic biodegradability test	Anoxic biomineralizable
UV/ozone or UV/H_2O_2	Abiotic photolabile
Permanganate/dichromate, ozone or hydrogen peroxide	Abiotic oxidative

These methods can be used to develop integrated fractionation schemes which can be used to assess the environmental impact of AOX/EOX in aqueous samples. Depending on the desired complexity, such schemes can either be comprehensive or can be simplified to make them more suitable for routine applications. We here propose both a comprehensive and a simplified scheme.

4.1 Proposed comprehensive fractionation scheme

The integrated fractionation scheme consists of several steps and is divided into two parts: a non-reactive and a reactive one. The non-reactive scheme is based on some of the physical chemical characteristics as indicated in Table 2. The scheme investigating degradation, however, is based on mineralization of the covalently bound halogens in the AOX/EOX mixture. The AOX/EOX value itself directly measures this. Apart from the halogen, carbon is also present in AOX/EOX, but once halogens have been removed, mineralisation of the carbon component is not relevant to investigation of the environmental impact of organohalogen mixtures.

Scheme 6 shows the first step of such a fractionation scheme which combines a number of aspects from the literature. The main parameters and characteristics of the substances are indicated in bold.

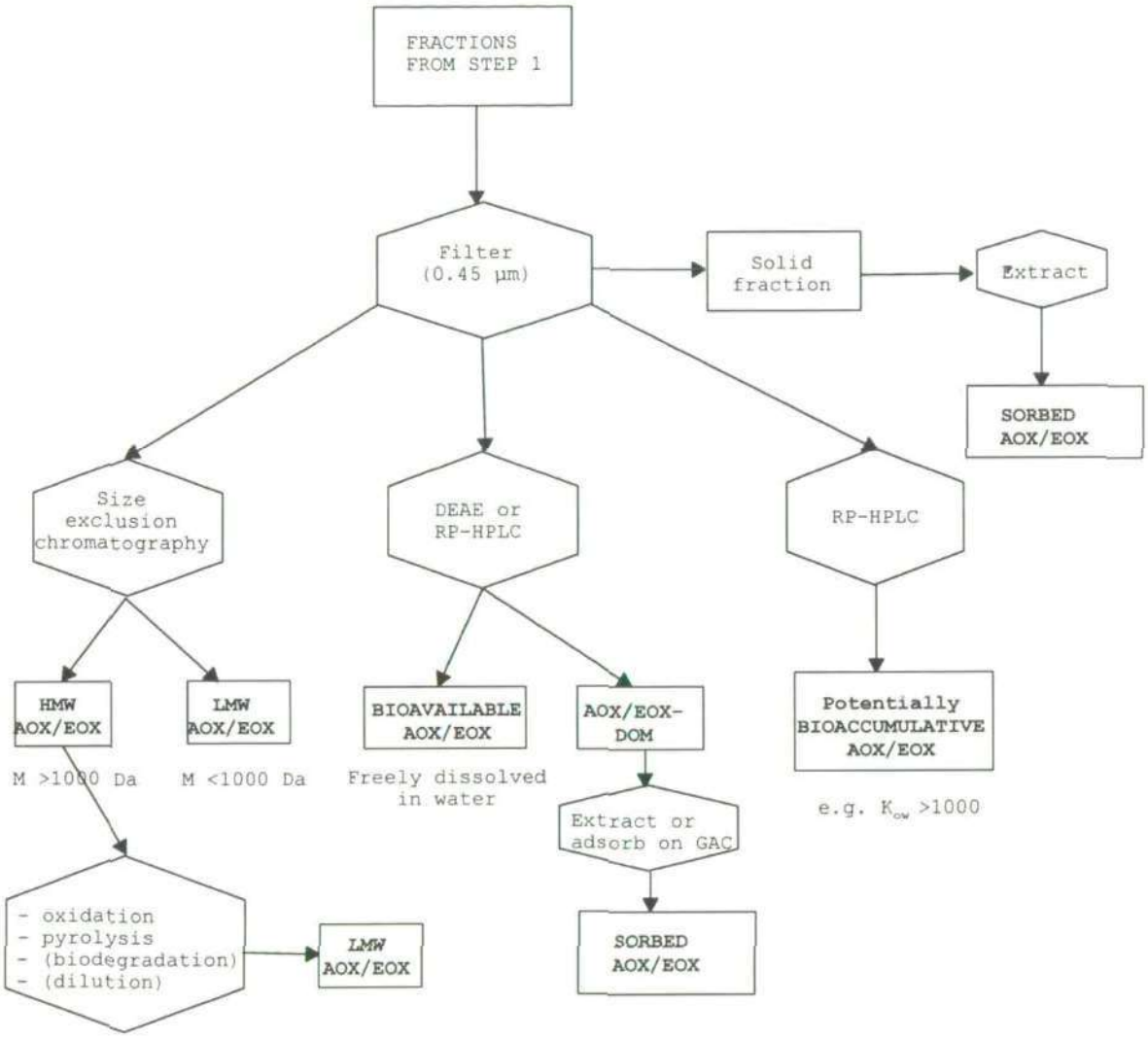


Scheme 6 A first step in a larger fractionation scheme to separate the AOX/EOX mixture into environmentally important fractions.

The different pH fractions may be fractionated further on the basis of bioavailability and bioaccumulation. A scheme which leads to several defined fractions is shown in step 2 (Scheme 7). This scheme is based on the assumption that no degradation takes place (i.e. persistence). This might be difficult to achieve especially if abiotic degradation mechanisms are involved. Biotic degradation can be excluded more easily, especially if toxic solvents are used. Reversed phase HPLC can be used to fractionate the bioaccumulative fraction either in parallel to or after separation of freely dissolved AOX/EOX from the sorbed fraction.

However, each fractionation method in this scheme has some drawbacks. Size exclusion chromatography separates the mixture according to molecular weight or size but the HMW fraction contains micelles and is thus not well defined. The formation and structure of these micelles has recently become of interest since they contain the strongly hydrophobic molecules. Dilution of this fraction may lead to a greater bioavailability of toxic substances. This also poses some questions on the bioavailability of the HMW fraction since it dissociates easily into smaller structure on dilution. Since the HMW fraction is in general of biotic origin, it is likely that this fraction is biodegradable, albeit slowly. This fraction may, therefore, become an important source of the LMW AOX/EOX fraction The behaviour of the HMW fraction should therefore be a point of investigation. Complete separation of DOM from the filtrate is, as indicated earlier in this report, difficult to achieve. The use of DEAE

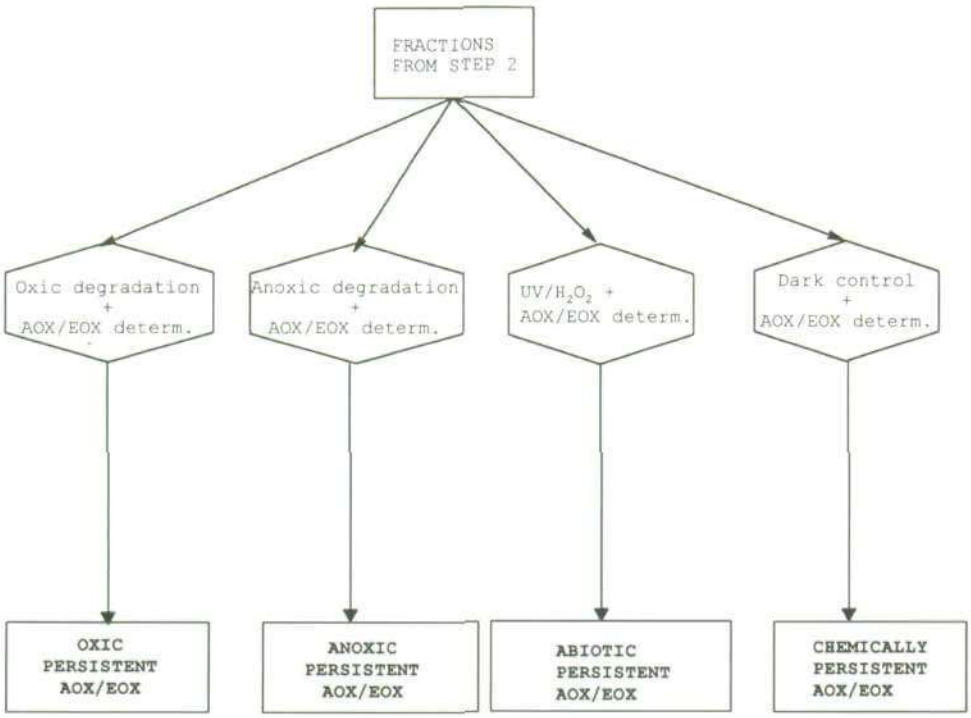
seems to lack a firm scientific basis while the use of RP-HPLC (Landrum et al., 1984) may lead quickly to breakthrough of the column.



Scheme 7 A second step in a larger fractionation scheme to obtain several defined environmentally important fractions of AOX/EOX.

The AOX/EOX value of each fraction can be determined. In this case it should be worthwhile to look for a possible mass balance in AOX/EOX. This indicates how successful the fractionation scheme functions. It also may indicate if the fraction AOX/EOX varies widely in time or not or other time trends may become visible e.g. the fraction volatile AOX/EOX increases if the total AOX/EOX value decreases or vice versa.

In the following step, step 3 (Scheme 8), (bio)degradable and persistent fractions are isolated.



Scheme 8 A third step in the AOX/EOX fractionation scheme to define environmentally important reactive fractions.

Based on the discussion of biodegradation in chapter 3, the following characteristics of the reactive fractions may be investigated: C:Cl ratio, relative rate, main characteristics of the persistent organohalogens (Table 3).

TABLE 3 MAIN CHARACTERISTICS OF ENVIRONMENTALLY IMPORTANT REACTIVE FRACTIONS

CHARACTE-RISTICS	OXIC DEGRADATION	ANOXIC DEGRADATION	ABIOTIC DEGRADATION	CHEMICAL DEGRADATION
C:Cl ratio	~ no change/ decrease	increase	increase	reaction dep.
Relative rate	fast	slow	variable	substance dep.
Organic part OX	oxidised	intact	depends	intact
Persistent Oxs	rel. highly chlorinated	rel. lowly chlorinated	rel. lowly chlorinated	reaction dep.

In this case it is assumed that (bio)degradation takes place in terms of disappearance of the reactants. However, in the case of AOX/EOX it is more logical to look at halogen mineralization. This may be measured directly in terms of AOX/EOX. The mineralization of carbon can only be measured if at the beginning the total carbon content in the sample is determined as well. Some OECD biodegradation tests, such as the OECD tests 301 B and C (OECD, 1993) could be adapted and used to measure the halogen mineralisation of the AOX/EOX mixture. A standardised anoxic biodegradation test method is in preparation. The outline of this method has been described recently (Nuck et al, 1996). This outline also describes (anaerobic) mineralization and should fit well in the scheme described above.

The abiotic degradation experiments are based on light and hydrogen peroxide. Such a combination is often used in water purification experiments. In this example, however, the light source should consist of a wavelength larger than 290 nm in order to match normal sunlight.

4.2 *Proposed simplified screening fractionation scheme*

The fractionation schemes described above can be used for detailed characterisation of AOX or EOX in environmental fractions. However, these schemes are too complex to be used for more routine purposes and for the comparison of sources. Neither do they provide easy means to assess the environmental impact of sources in terms of persistent, bioaccumulative and toxic (PBT) compounds. Therefore, a simplified scheme is proposed in this section which is more suitable for the rapid assessment of PBT organohalogens in AOX/EOX-containing waste water.

The philosophy behind this scheme is that the AOX/EOX fraction that has an impact on the aquatic environment in general and the marine environment in particular is the one of concern to DIFF. Therefore, in the proposed scheme a series of fractionations is applied which has been chosen to reflect the processes undergone before and after the waste water is discharged into the aquatic environment. Firstly, these processes include sorption, biodegradation, and volatilisation. These processes will lead to removal of part of the organohalogens initially present in waste water, which either will not enter the environment or will be degraded to inorganic halides in it. Therefore, organohalogens which are volatile, strongly sorbed, intrinsically biodegradable, not highly lipophilic and not bioavailable are removed in this scheme. The persistent fraction which remains in the water phase is then selected for the bioavailable and bioaccumulative organohalogens, yielding the potentially persistent and bioaccumulative AOX/EOX (pPB-AOX/EOX) fraction. These parameters are generally accepted as the most important ones for environmental risk assessment. As far as possible, the steps involve the use of well developed standard methods such as those in the OECD Guidelines (OECD, 1993). In each step, AOX (or alternatively EOX) can be applied as analytical technique. Because of the use of standardised methods, this is a practical, flexible scheme.

The pPB-AOX/EOX fraction can be used not only as an estimate of the possible environmentally important fraction of the organohalogens present in the sample, but can also be subjected to further characterisation to assess the environmental impact of this fraction and to identify important components.

This scheme is focused in particular on AOX/EOX entering the aquatic environment via effluents from sewage treatment plants, with the biodegradation step at the beginning of the scheme. In other cases (e.g. where direct discharge to surface water takes place) it could be important to know more about the original emissions of organohalogens. The scheme could be adapted by moving the sorption and biodegradation steps to after the fractionation of the pPB-AOX/EOX fraction.

The scheme proposed here differs from the TIE-based schemes such as those used by the EPA in that these use toxicity determinations as starting points, whereas in the scheme proposed here the pPB-AOX/EOX fraction is derived first and toxicity is tested subsequently.

It should be noted that a scheme such as that proposed can be used for both AOX and EOX as analytical parameters to yield pPB-AOX or pPB-EOX fractions and could also be adapted for nonhalogenated organic contaminants. In this case the scheme bears some resemblance to the Whole Effluent Environmental Risk (WEER or TEM) method developed recently by the Dutch Institute for Inland Water Management and Waste Water Treatment (RIZA) (Tonkes and Baltus, 1997).

The proposed fractionation scheme consists of the following steps:

Volatilisation

The first step is to remove the volatile AOX/EOX by means of purging. Considering the possibility of reactions of AOX/EOX with oxygen to form more volatile products, as discussed in chapter 3, purging with nitrogen would be the preferred technique. This is a relatively simple technique which could be applied routinely.

Sorption

Two approaches could be used to distinguish sorbed and non-sorbed AOX/EOX. One possibility would be to remove particulate matter and the AOX/EOX sorbed to this material from the sample by filtration. An alternative would be to remove potentially sorbing compounds by exposing the aqueous sample to suspended sediment, according to for example the OECD test method 106, followed by the removal of the sorbed fraction with the sediment. However, the potentially sorbing fraction will consist at least in part of hydrophobic compounds which are also potentially bioaccumulative. It is therefore likely the use of the second approach would lead to removal of potentially bioaccumulating compounds and thus an underestimation of the PPB-AOX/EOX fraction. We therefore propose that only the AOX/EOX fraction actually present in the sorbed phase be distinguished by removal of the particulate phase using filtration. Such a filtration step can be applied very readily to waste water samples. As discussed in Chapter 3, this approach assumes that the sorbed fraction is not potentially bioavailable and therefore does not have an impact on the aquatic environment. Indirect effects arising after desorption of the initially sorbed fraction in the aquatic environment are therefore ignored.

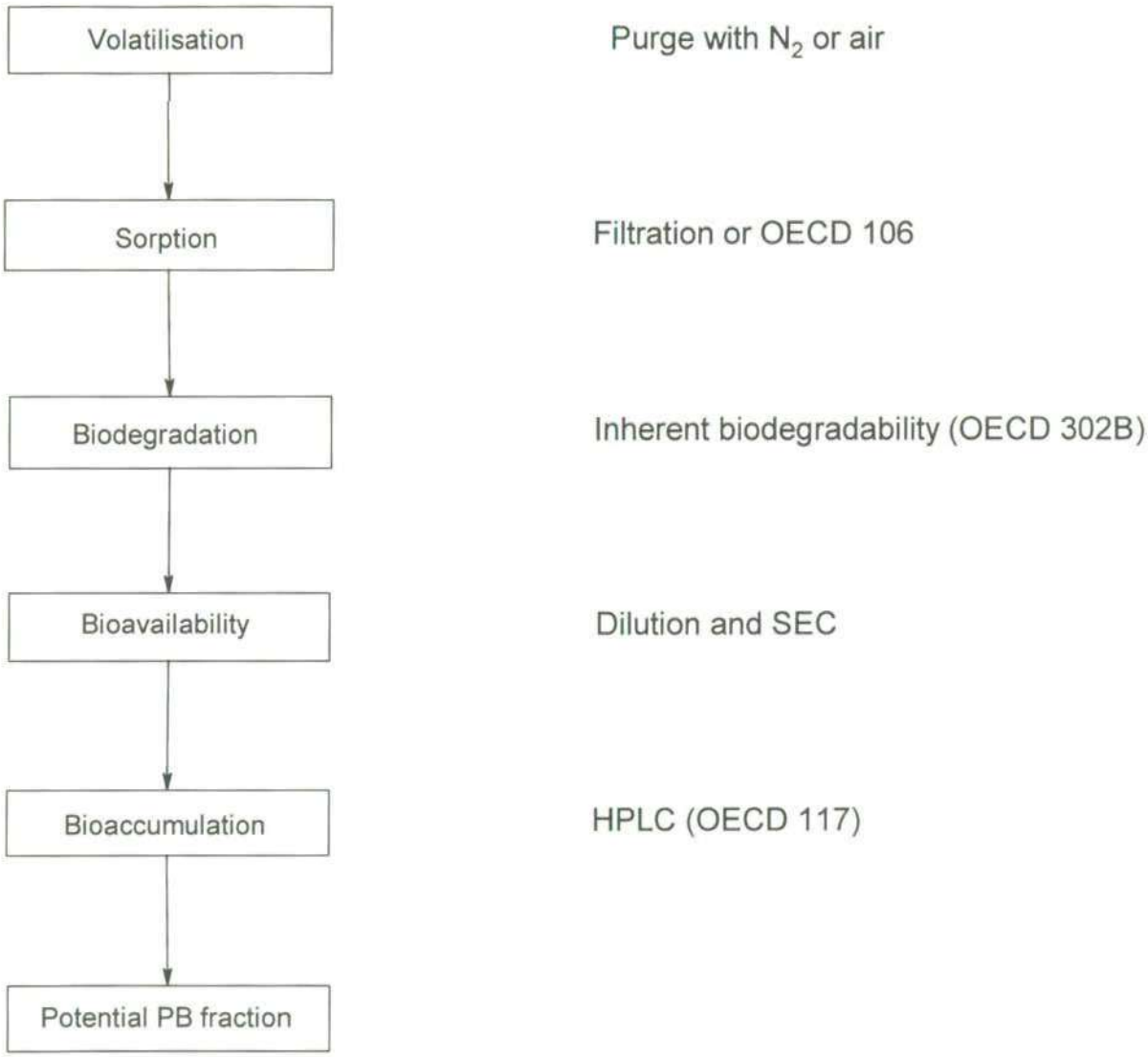
Biodegradation

The biodegradable fraction of AOX/EOX is removed in the following step. As proposed in chapter 3, this could be done using one of the generally accepted standard test methods in which mineralisation is tested, such as the OECD methods (OECD, 1993), although it is only mineralisation of covantly bound halogens, not carbon, that is of interest here. The actual choice of method may be determined by circumstances. In all cases AOXor EOX would be used as analytical parameter which, as mentioned earlier, gives the non-mineralisable fraction of organohalogens. This step is intended to model biodegradation in oxic environments in general - in sewers, sewage treatment plants and the aquatic environment.

The ready biodegradability tests such as the closed bottle test (OECD 301D) are readily applicable, but may underestimate the biodegradable fraction under real world environmental conditions. A more realistic assessment of the biodegradable fraction may be obtained with one of the tests for inherent biodegradability. These tests use much higher biomass concentrations and longer incubation times than the ready biodegradability, thereby increasing the potential for adaptation to an initially poorly biodegradable compound. One of these test is the Zahn-Wellens test, OECD 302B. Alternatively, untreated effluents could be tested using a waste water treatment simulation test such as the Semi-Continuous Activated Sludge test (OECD 302A) to determine the fraction AOX/EOX removed in the treatment process.

To simulate biodegradation of AOX/EOX after emission to surface water, the new test method using surface water as an inoculum which was developed by Aquasense for the WEER or TEM method (Aquasense, 1995) could be applied in this scheme.

Losses of AOX/EOX in the biodegradation tests as a result of sorption to sludge or volatilisation of volatile intermediates should be quantified for mass-balance purposes.



Bioavailability

The bioavailable fraction is distinguished by removing the high molecular weight fraction of organohalogens as well as the fraction bound to high molecular weight DOC. The most suitable techniques for this operation are filtration with a size-exclusion membrane and size exclusion chromatography, as described in chapter 3. Because of the concentration-dependent aggregation of high molecular weight materials (also described in chapter 3), it is recommended that water samples be diluted before this fractionation step to more closely represent the situation in the environment. However, it is possible that the biodegradation test will have diluted the original sample sufficiently to account for this effect. This could be checked by measuring any change in HMW:LMW ratio (of AOX/EOX) on further dilution.

Bioaccumulation

The bioaccumulative fraction of AOX/EOX can be fractionated on the basis of octanol-partition coefficient (K_{ow} or P_{ow}), which can be most conveniently determined using the reversed-phase HPLC method (OECD method 117, OECD, 1993). This method also yields a fraction which can be used for further characterisation. To take account of bioaccumulation of organic acids and bases, it is likely that this fraction should be performed at different pHs.

Fractionation on the basis of pH adjustment is not included in this scheme, as this is generally applied to the characterisation of the different chemical species present and gives little direct information on the environmental impact of AOX/EOX.

Order of the fractionation steps

The proposed order in which the fractionation steps would be carried out usefully parallels the order of processes that AOX/EOX in waste water would undergo in the environment. Alternative sequences are possible, in particular those in which the biodegradation step is moved. Sorbed materials which are not biodegraded are removed with the sludge. As far as the biodegradation step is concerned, the advantage of the proposed sequence is that products of incomplete biodegradation of AOX/EOX forming bioavailable products from large, non-bioavailable, molecules, are formed before the bioavailability and bioaccumulation steps. In this way, the scheme takes the impact of biodegradation on bioavailability and bioaccumulation of AOX/EOX into account. As mentioned above, in cases where this is not required, for example where assessment of the complete AOX/EOX is aimed at, the biodegradation step could be omitted completely. Similarly, the removal of the sorbed fraction could be omitted if this fraction is required in the complete assessment.

The separation of the bioaccumulative fraction of AOX/EOX is placed at the end of the scheme to avoid the solvents used in the HPLC fractionation interfering with the biodegradability tests and to account for changes in log K_{ow} distribution caused by biodegradation.

This sequence of fractionation steps in this scheme should relatively easily yield the potentially persistent and bioaccumulative AOX/EOX (pPB-AOX/EOX) fraction. This is the fraction which may have an environmental impact. The concentration of the pPB-AOX/EOX fraction could be used to calculate an estimated annual emission which could be used to prioritise waste water for further investigation.

Further characterisation

If the estimated emission of the pPB-AOX/EOX fraction indicates that further work is necessary, a detailed assessment of its environmental hazard should be performed. It should be recognised that the pPB-AOX/EOX fraction consists of potentially persistent and bioaccumulative compounds. Further characterisation should give more information on the extent to which this fraction is indeed persistent and bioaccumulative. For example, other degradation mechanisms, such as anaerobic biodegradation or abiotic degradation, may play an important role in reducing the environmentally persistent fraction.

Most importantly of course, further studies should include toxicity studies. In addition, toxicity tests coupled to chemical characterisation steps such as those described in chapter 3 could lead to the identification of organohalogen compounds responsible for the effects. However, a means is required to separate the toxic effects of organohalogens from those of other, non-halogenated, compounds which are present in the pPB fraction. Methods used in the TIE approach may be useful here. On the other hand, the fractions may at this

point be sufficiently clean to enable separation and identification of individual compounds. Toxicity data may be available for these compounds or their toxicity may then be tested individually. The test methods should not only investigate the potential acute toxicity, but should also include methods for chronic toxicity and mutagenicity. Examples of suitable tests are those used in the WEER or TEM method (Tonkes and Baltus, 1997).

These further fractionation and characterisation steps would use less well established methods and would therefore not be suitable for routine applications. These factors can best be assessed using purposely designed studies. Furthermore they are most relevant for processes occurring after discharge of effluents to the environment. Excluding these further steps from the basic scheme keeps it focused and practical.

Further work is required before this scheme can be applied in practice. The proposed integrated scheme should be fully validated, the limits of detection and quantification and the mass balance determined. The scheme should be tested with waste water from different sources and synthetic mixtures of known pPB organohalogens. Methods for the further characterisation of the pPB-AOX/EOX fraction need to be developed. In particular methods to account for degradation processes other than aerobic biodegradation. Also required are methods to account for the possible interference of nonhalogenated compounds in the toxicity tests (acute, chronic and mutagenic) done on the pPB-AOX/EOX fractions.

Despite using standard techniques to remain relatively simple, this scheme is unlikely to be suitable for routine monitoring purposes in which pPB-AOX/EOX would for example be used as a regulatory parameter. This scheme is however suitable for the evaluation of the impact of AOX/EOX in waste water on the aquatic environment, for the identification of priorities for further investigation and remediation and for the isolation of hazardous compounds in AOX/EOX.

5 CONCLUSIONS AND RECOMMENDATIONS

The aim of this study was to search the literature for methods which were suitable for assessing the environmental impact of AOX/EOX in waste water and enabled the impact to be expressed as an environmentally relevant sum parameter. Such a parameter would give a better indication of environmental impact than just the concentration of AOX/EOX would. Preferably these methods should be coupled to fractionation methods which would give more information on groups of environmentally hazardous organohalogen components present in water samples.

The literature contains many reports of fractionation procedures carried out on various organohalogen mixtures, though most are aimed at identifying or characterising individual compounds from specific waste water sources. They do not offer any real prospect of being able, even in combination, to identify all the substances present in different waste waters, nor do they generally provide good data on the environmental properties of these substances. Furthermore, these methods are often poorly characterised in terms of recoveries of the individual fractionations and the mass balance of the scheme as a whole.

Some fractionation schemes do, however, use techniques which correlate with some aspects of the environmental behaviour or fate, and so yield fractions with specific environmental relevance. By combining these schemes, which examine properties such as volatility, sorption (non-bioavailability), degradability and potential for bioaccumulation, it has been possible to construct a relatively comprehensive scheme (Chapter 4.1), which could progressively examine these aspects. The difficulty is that such a scheme would create many different fractions, many of which overlap, so no clear picture is available of the quantities of substances present which may have combinations of properties that might make them environmentally hazardous.

However, since the compounds of greatest, long-term, environmental concern are those which would be persistent, bioaccumulative and toxic, a simplified scheme (Chapter 4.2) has been devised which would initially isolate any compounds which might be persistent and bioaccumulative in a single (pPB) fraction. The actual environmental hazard of this pPB fraction will depend also on the toxicity of this fraction and on the role played by other environmental fate processes not covered by the scheme. Therefore, further characterisation of this fraction should include extra testing for abiotic degradability, anaerobic biodegradability, mutagenicity, acute and chronic toxicity.

However this simplified scheme could be used as an efficient screening scheme to identify just those waste waters which might have a significant PBT organohalogen content. By screening and prioritising waste waters for further study, this scheme offers, once fully developed and validated, a practical approach to help in environmental and regulatory decision-making. It is not, however, envisaged that the scheme would be rapid enough to be used for frequent measurements for routine monitoring purposes.

If it were decided to develop this simplified scheme as a broadly-applicable approach to assess the potential environmental impact of organohalogen-containing waste waters, further work would be required to develop, test and validate the procedures, particularly:

a. Selection and trials of methods

Specific methods, and ideally (as in the TEM/WEER approach for studying Whole Effluent Environmental Risk of complex effluents) standard analytical methods need to be

chosen for each step. They should be tested both separately and in combination on a representative range of waste waters.

b. Formal analytical validation

The scheme then needs to be formally analytically validated, using waste waters containing spikes of known compounds and by drawing-up mass balances to establish recoveries and to determine the limits of detection and of quantification.

c. Further development

Further development work would also be needed on:

- Methods to assess the anaerobic biodegradation and abiotic degradation of the pPB-AOX/EOX fraction.
- The feasibility of identifying specific substances in the potentially less complex pPB fraction.
- Selecting and developing a range of methods, bearing in mind the need to separate the toxic effects of organohalogens from those of non-halogenated compounds which will also be present in the pPB-AOX/EOX fraction.

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7 LIST OF ABBREVIATIONS

AOX	Adsorbable Organic Halogens (adsorbed onto GAC)
BAF	BioAccumulation Factor (conc. ratio in organism and defined phase)
BCF	BioConcentration Factor (conc. ratio in organism and water)
BKME	*Bleach Kraft Mill Effluent*
BOD	Biological Oxygen Demand, eg. according to OECD test 301 C.
(D)CI	(Double) Chemical Ionisation (method to form ions for detection in MS)
CLOGP	Calculation method for log K_{ow} developed by Hansch and Leo
COD	Chemical Oxygen Demand
DEAE	DiEthylAminoEthyl Cellulose
DOM	Dissolved Organic Matter (fraction after filtration through 0.45 μm)
DS	Detection System (library systems of peak degradation by MS)
E	Redox potential
EC_{50}	Concentration at which 50% effect is found
ECF	Elemental Chlorine Free
EDTA	EthyleneDiamineTetraAcetic acid (a chelating agent for metal-ions)
(US)EPA	Environmental Protection Agency (in the US)
EI	Electron ionisation (ionisation method used in MS)
EOCL	Extracted Organo Chlorides
EOX	Extractable Organic Halogens
EPOCL	Extractable Persistent Organic Chlorides (fraction EOX remaining after using concentrated sulphuric acid)
GAC	Granulated Active Carbon (Adsorption substance used in AOX det.)
GC	Gas Chromatography
GC-(HR)MS(D)	GC coupled to a (High Resolution) Mass Spectrometer Detector
GC-AED	GC coupled to an Atomic Emission Detector
GC-ECD	GC coupled to an Electron Capture Detector
GC-ELCD	GC coupled to an Electrolytic Conductivity detector
GC-FID	GC coupled to a Flame Ionisation Detector
GC-NPD	GC coupled to a Nitrogen and Phosphor Detector
GCB	Graphitized Carbon Black (A solid adsorption substance)
GPC	Gel Permeation Chromatography
HAA	Halogenated Acetic Acids
HLC	Henry Law's Constant (equilibrium constant of a substance "dissolved" in the gas phase and water)
HMW	High Molecular Weight fraction (> 1000 Da)
HPLC	High Performance (Pressure) Liquid Chromatography
K_{ow}	Octanol-water partition coefficient
LC-MS	Liquid Chromatography coupled to MS
LLE	Liquid-liquid Extraction (extraction of substances from the *liquid phase with a solvent)*
LMW	Low Molecular Weight fraction (< 1000 Da)
NAA	Neutron Activation Analysis
NMR	Nuclear Magnetic Resonance (used for identification purposes)
N-PAH	Poly Aromatic Hydrocarbons containing a nitrogen atom .
OECD	Organisation for Economic Co-operation and Development

OX	Organohalogen
PAC	Poly Aromatic Compounds
PCB	Poly Chlorinated Biphenyl
POX	Purgable Organo Halogens
QSAR	Quantitative Structure-Activity Relationship
RP-HPLC	Reversed phase-HPLC (the column used is apolar)
SEC	Size Exclusion Chromatography
SIM	Single Ion Monitoring (mode used for MS)
SPE	Solid Phase Extraction (extraction of substances by a solid)
TCF	Total Chlorine Free
TI	Toxicity Index
TIE	Toxic Identification Evaluation
TLC	Thin Layer Chromatography
TOC	Total Organic Carbon
TOX	Total Organic Halogen
UV	Ultra Violet light
VOC	Volatile Organic Carbon
VOX	Volatile Organic Halogen
XAD	(Type of resin used in SPE)

8 APPENDIX

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