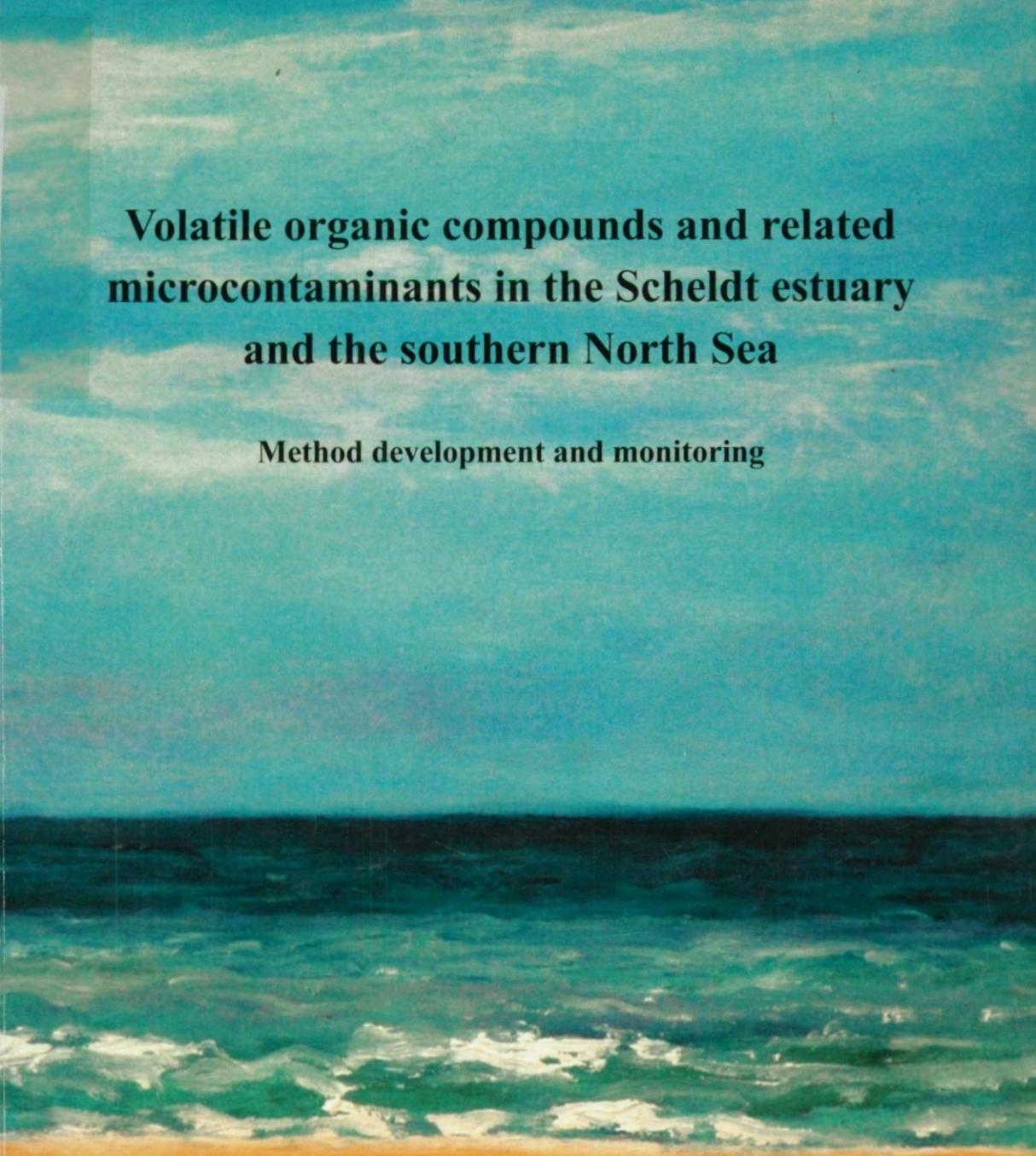
Volatile organic compounds and related microcontaminants in the Scheldt estuary and the southern North Sea

Method development and monitoring



Patrick Roose

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VRIJE UNIVERSITEIT

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door

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Aan mijn ouders

This study was carried out at the Sea Fisheries Department, Centre for Agricultural Research during the period 1995-1999 and at the Management Unit Mathematical Models of the North Sea of the Royal Belgian Institute for Natural Sciences during the period 1999-2005.

Contents

1 Introduction

1 MONITORING OF ORGANIC MICRO-CONTAMINANTS IN THE MARINE ENVIRONMENT: PRINCIPLES, PROGRAMMES AND PROGRESS MADE*

1.1 General introduction

After the Second World War, modern society rapidly evolved through a technological progress that seemed to open virtually unlimited opportunities. It was especially the chemical industry, which delivered new products at a breathtaking pace. Unfortunately, this growing economy also caused increasing and widespread pollutant emissions, a problem that was previously mostly limited to the immediate vicinity of emission sources. The general awareness of the potential danger of large-scale contamination increased, as a number of incidents gave global prominence to the potential dangers of this evolution. In Minamata, Japan (1961) a crippling, sometimes fatal, disease was found to be related to industrial mercury discharges. Scientists discovered that even in the open ocean, big fish sometimes contained high concentrations of mercury. In about the same period, the pesticide DDT proved to be responsible for the decline of bird of prey populations, such as the bald eagle in North America [1] and the white-tailed eagle in the Baltic [2]. Aided by novel analytical techniques such as gas chromatography, scientists started investigating their environment for the presence of DDT and its degradation products. In 1966 Sören Jensen [2], while measuring DDT, identified a number of unknown peaks in his chromatograms as polychlorinated biphenyls (PCBs). The same compounds were found to be the actors in the Yusho incident in Japan, in 1968, where a massive accidental exposure of humans to PCBs and trace levels of PCDFs (polychlorinated dibenzofurans) occurred, caused by ingestion of a commercial brand of rice oil contaminated with these chemicals. As a result, about 1800 patients showed clinical symptoms such as acneiform eruptions, pigmentation of the skin, nails, and conjunctivas, increased discharge from the eyes, and numbness of the limbs [4]. Later work showed that these, and many other, xenobiotics are present in all compartments of the environment, even in regions far away from known sources, such as the Arctic and Antarctic [5]. In this way it became apparent that these modern chemicals, their breakdown products and by-products generated during their production, can threaten the marine environment.

* For a glossary of the most important abbreviations used, see 1.6 end of chapter.

Soon after having realized the extent of the potential danger, governments started to take measures to reduce or eliminate the release of contaminants to the environment. Limiting ourselves to the above examples, today the EU has extensive legislation concerning mercury and its compounds, which includes restrictions on marketing and use, a ban on use of certain products and applications, regulations concerning emissions to air and water, and waste treatment legislation [6]. Similar measures have been taken in the US [7]. As a result of the link between thin eggshells and DDT concentrations in eggs, DDT was banned in the US in December 1972 [8]. In Europe, DDT was partly banned for agricultural uses in December 1978 (Directive 79/117/EEC) while a total ban for agricultural uses occurred in March 1983, with Directive 83/131/EEC. Today, DDT is only used in the EU as an intermediate in the production of the pesticide dicofol, where it is handled in closed production systems. In 1976 the Toxic Substances Control Act led to a ban on the production of PCBs in the US. The use of PCBs in open applications such as printing inks and adhesives was banned in the European Community in 1976 under Directive 76/403/EEC. The use of PCBs as a raw material or chemical intermediate was banned in the EU in 1985 (85/467/EEC, 6th amendment to Directive 76/769/EEC).

Table 1.1: Overview of major long-term monitoring programmes, and the contaminants and matrices measured.

Organisation or programme	Start of the programme	Parameters[2,3]	Sample type
AMAP	1978	HM, PCBs, PAHs, OCPs	biota, sediment, water
HELCOM	1979	HM, PCBs, PAHs, OCPs, OTINs	biota, sediment
NS&T	1986	HM, PCBs, PAHs, OCPs	biota, sediment
IMW[1]	1965	HM, PCBs, PAHs, OCPs	biota (molluscs)
OSPAR	1978	HM, PCBs, PAHs, OCPs, OTINs	biota, sediment

[1] The IMW actually started in 1991-1992, but data were already available from earlier programmes with a different name as early as 1965. [2]Not all parameters measured during entire period; [3] HM, heavy metals; PCBs, polychlorinated biphenyls; PAHs, polycyclic aromatic hydrocarbons; OCPs, organochlorine pesticides; OTINs, organotins.

The realisation of the potential danger of certain, or rather, many chemicals did not only result in a call for measures to regulate their input into the seas, but also in a call for long-term monitoring. National and international workshops and study groups were convened to discuss the monitoring and assessment of microcontaminants and to outline strategies for terrestrial and aquatic programmes [9].Monitoring has now been ongoing for several

decades and has revealed the ubiquitous presence of trace organic microcontaminants and heavy metals in all compartments of the environment. Table 1.1 gives an overview of some well-known, long-term marine monitoring programmes and the principal contaminants that are being measured. They will be discussed in greater detail below.

Although heavy metals are important contaminants of the marine environment, in the present chapter we will primarily discuss organic micropollutants (OMPs). The OMPs still are a major cause of concern for the marine environment, despite the measures that have been taken to reduce their input and/or use. For example, in the OSPAR Quality Status Report 2000, Region II Greater North Sea [10], next to fisheries and nutrients, the OMPs are considered as a first-priority-class human pressure on the North Sea. Although the concentrations of some pollutants are decreasing, an increasing number of xenobiotics can be detected [10]. There is, however, a general lack of data on the presence of organic hazardous substances in the marine environment. The report recommends that steps should be taken to close the gaps in knowledge, "… in particular regarding the occurrence and effects of hazardous substances in the marine environment". The main reasons why knowledge on the occurrence of OMPs is patchy, are that (1) most projects focus on just a single group of chemically related pollutants; (2) all relevant compartments within a study area are not always investigated; (3) selection of sampling sites is not coordinated in most monitoring programmes and (4) analytical methods are not always developed to the level that allows measurement of the target compounds in marine field samples at suitably low concentrations [10, 63].

This chapter aims at giving an overview of the monitoring activities that have been carried out in the framework of marine pollution by organic compounds. The programmes, their efforts, successes and shortcomings will be discussed. When referring to 'monitoring', the OSPAR definition [11] will be applied: "the repeated measurement of (1) the quality of the marine environment and each of its compartments, *viz.* water, sediment and biota; (2) activities or natural and anthropogenic inputs which may affect the quality of the marine environment; (3) the effects of such activities and inputs."

As regards 'pollution', this is defined by OSPAR as "the introduction by man, directly or indirectly, of substances or energy into the maritime area which results, or is likely to result, in hazards to human health, harm to living resources and marine ecosystems,

damage to amenities or interference with other legitimate uses of the sea" [11]. The EU definition is "the direct or indirect introduction, as a result of human activity, of substances or heat into the air, water or land which may be harmful to human health or the quality of aquatic ecosystems or terrestrial ecosystems directly depending on aquatic ecosystems, which result in damage to material property, or which impair or interfere with amenities and other legitimate uses of the environment" [12].

The discussion will mainly focus on monitoring activities in the northeast Atlantic and, more specifically, the North Sea. Where appropriate, other areas will be discussed.

1.2. Monitoring programmes for the marine environment

It is not the intention to give an exhaustive overview of all monitoring programmes for OMPs that are currently active, but to briefly describe a number of major programmes (*cf.* Table 1.1 and see Figure 1.5 below) and to consider both common aspects and mutual differences. The programmes that are relevant for the northeast Atlantic and the North Sea will be discussed in greater detail. In essence, monitoring is about gathering information – information that allows authorities to tentatively assess the quality of the environment, to recognise threats posed by human activities and to assess whether earlier measures have been effective. It is precisely here that lays the challenge for a monitoring programme: will the data that have been obtained, be practically useful.

1.2.1. Monitoring programmes for the North Sea area

The Oslo and Paris Commission and the Joint Assessment and Monitoring Programme

When monitoring and assessment of the quality of the marine environment in the northeast Atlantic are being reviewed, it cannot be done without taking its chief actor into consideration, the Oslo and Paris Commission (**OSPAR**).

In 1974, the 1972 Oslo Convention – also called the Convention for the Prevention of Marine Pollution by Dumping from Ships and Airplanes, entered into force. The Convention regulated dumping operations involving industrial waste, dredged material and sewage sludge (North Sea Task Force, 1993). The Convention was signed by BE, DK, FI, FR, DE, IS, IE, NL, NO, PT, ES, SE and the UK (ISO 3166 codes for countries). Although most of the activities mentioned in the initial convention, such as waste disposal

and combustion at sea, have been discontinued, the guidelines and approach paved the way for further work. The Paris convention, or Convention for the Prevention of Marine Pollution from Land-Based Sources was established in 1974 and came into force in 1978. BE, DE, DK, ES, FR, IE, IS, NL, NO, PT, SE, UK, and the Commission of the European Communities (EC) signed the Convention. Its principal aim was to prevent, reduce and, if necessary, eliminate pollution of the Convention area from land-based sources, which are discharges from rivers, pipelines, the coast, but also offshore installations and the atmosphere [13].

The tasks set forth in both Conventions were originally handled by two individual commissions (Oslo commission and Paris commission). In 1978, both commissions established a Joint Monitoring Programme, the JMP, obliging contracting parties to initiate monitoring activities for a number of parameters in their water bodies. Among these parameters were heavy metals and PCBs. The results of the measurements were to be reported to the International Council for the Exploration of the Seas, ICES, where they would be processed and statistically analysed.

ICES, established in 1902, claims to be the oldest intergovernmental organization in the world concerned with marine and fisheries science [14]. ICES is a leading scientific forum for the exchange of information on the sea and its living resources, and for the promotion and coordination of marine research by scientists in its member countries. Since the 1970s, a major area of ICES work has been to provide information and give advice to member country governments (BE, CA, DE, DK, EE, ES, FI, FR, IE, IS, LV, NL, NO, PL, PT, RU, SE, UK, and the US) and international regulatory commissions on the protection of the marine environment and for fisheries conservation. In support of these activities, the ICES Secretariat in Denmark maintains three databanks – the oceanographic databank, the fisheries databank and the environmental (marine contaminants) databank. The Advisory Committee on the Marine Environment (ACME) is the Council's official body for the provision of scientific advice and information on the marine environment, including marine pollution, to member countries, other bodies within ICES, and relevant regulatory commissions.

In the eighties, the policy of the Oslo and Paris Commissions evolved with the general evolution of environmental policy in Western Europe, voiced at the Ministerial

Conferences for the Protection of the North Sea [15]. For instance, at the 1987 conference in London, all North Sea states accepted the 'precautionary principle', which says that "the basis of action in regard to the reduction of inputs of substances that are persistent, toxic and liable to bioaccumulate should be based on the principle of 'precautionary action'" and that such inputs should be limited "by the use of the best available technology and other appropriate measures". The Paris Commission adopted this precautionary principle in 1989 and the principle of 'best environmental practice' for diffuse sources in 1991. It was soon recognised that the existing Oslo and Paris Conventions did not adequately control some of the many sources of pollution, and that a revision was warranted. This should address all sources of pollution of the marine environment and the adverse effects of human activities upon it, taking into account the precautionary principle and strengthening regional cooperation. This resulted, not in a revision of the initial conventions, but more importantly, in the merger of both commissions into a new convention, the Convention for the Protection of the Marine Environment of the North-East Atlantic or OSPAR [16].

Figure 1.1: OSPAR Convention area and member countries

The new Convention was opened for signature at the Ministerial Meeting of the Oslo and Paris Commissions, Paris, 21-22 September 1992. The Convention has been signed by all contracting parties (CPs) to the Oslo and to the Paris Conventions (BE, CH, DE, DK, ES, FI, FR, IE, IS, LU, NL, NO, PT, SE, UK, and the EC). After the ratification by all above-mentioned states and the EC, the Convention entered into force on 25 March 1998. The convention area is shown in Figure 1.1.

It is worthwhile to mention some fundamental principles that are recognised by the Convention and therefore, all CPs. These include:

- the vital importance to all nations of the marine environment and the fauna and flora which it supports,
- the inherent value of the marine environment of the North-East Atlantic and
- the necessity for providing coordinated protection for it,
- the essential importance of concerted action at national, regional and global levels to prevent and eliminate marine pollution and to achieve sustainable management of the maritime area – that is, the management of human activities in such a manner that the marine ecosystem will continue to sustain the legitimate uses of the sea and will continue to meet the needs of present and future generations,
- the desirability to adopt, on the regional level, more stringent measures with respect to the prevention and elimination of pollution of the marine environment or with respect to the protection of the marine environment against the adverse effects of human activities that are provided for in international conventions or agreements with a global scope, and
- the danger posed by pollution to the ecological equilibrium and the legitimate uses of the sea.

The CPs, therefore, have the general obligation to take all possible steps to prevent and eliminate pollution and will take the necessary measures to protect the maritime area against the adverse effects of human activities so as to safeguard human health and to conserve marine ecosystems and, when practicable, restore marine areas which have been adversely affected. Specifically with regard to hazardous substances, the objective of the Commission is to:

> "Prevent pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances, with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances".

The key objective of the strategy is the "cessation of discharges, emissions and losses of hazardous substances by 2020". The convention imposes on the OSPAR Commission (OSPARCOM) duties to define and implement collaborative monitoring programmes, to approve the presentation and interpretation of their results and to carry out [quality status] assessments, including in such assessments both an evaluation of the effectiveness of the measures taken and planned for the protection of the marine environment and the identification of priorities for action. The OSPAR Convention rightly requires the CPs, amongst other things, to "cooperate in carrying out monitoring programmes", and to develop quality assurance methods and assessment tools.

To monitor environmental quality throughout the North-East Atlantic, a Joint Assessment and Monitoring Programme (JAMP) has been established, which has recently been revised [17]. This JAMP has been built upon experiences gained through, amongst others, the former JMP and the Monitoring Master Plan of the North Sea Task Force. Under the JAMP umbrella, new guidelines and assessment tools have been, and are being, produced.

The main objectives of JAMP are (1) the preparation of environmental assessments of the status of the marine environment, the maritime area or its regions, including the exploration of new and emerging problems and (2) the preparation of contributions to overall assessments of the implementation of the OSPAR Strategies, including in particular the assessment of the effects of relevant measures on the improvement of the quality of the marine environment. Such assessments will help inform the debate on the development of further measures. These objectives are supported by the implementation of collective OSPAR monitoring, including the development of the necessary methodologies and the preparation of environmental data and information products needed to implement the OSPAR Strategies.

The above can be illustrated by having a close look at the Coordinated Environmental Monitoring Programme or CEMP [18]. The CEMP can be described as that part of monitoring within JAMP where the national contributions overlap and are co-ordinated. Three elements are essential for the realisation of CEMP – guidelines, quality assurance tools and assessment tools. These are presently available for certain JAMP issues but, more importantly, it means that common guidelines, quality assurance and assessment tools have to be in place before monitoring is undertaken. Also, CEMP is continuously under development, both with regard to the tools and the strategy and working schemes for particular issues, planning of activities in space and time, submission and management of data and identification of gaps in the coverage by CEMP.

In 1999, CEMP was adopted by OSPAR; it was last updated in 2004. It identifies a number of key parameters that are of particular concern to the marine environment. So far, the list includes the following parameters:

- mercury, cadmium and lead in biota and sediments
- PCBs in biota and sediments
- PAHs in biota and sediments
- nutrients in seawater
- direct and indirect eutrophication effects
- PAH- and metal-specific biological effects
- organotins in sediments and TBT-specific effects (from 2003 onwards).

However, this does not imply that these are the only chemicals that OSPAR considers to be important for the marine environment. The entire list and its selection procedure will be discussed later in this chapter.

The European Commission and the Water Framework Directive

Early European water legislation began in 1975 by setting standards for rivers and lakes used for drinking water abstraction and culminated in 1980 in setting binding quality targets for drinking water. At that time, it included legislation on quality objectives for fish waters, shellfish waters, bathing waters and groundwaters. The main emission control element was the Dangerous Substances Directive (76/464/EEC).

In 1988 the Frankfurt Ministerial Seminar on Water reviewed the existing legislation and identified a number of possible improvements and gaps. This initiated the second phase of water legislation, resulting in 1991 in the adoption of the Urban Waste Water Treatment Directive (91/271/EEC) – providing for secondary (biological) wastewater treatment, and even more stringent treatment where necessary – and the Nitrates Directive (91/676/EEC), which addressed water pollution by nitrates from agriculture. Other legislative results of these developments were EC proposals for action on a new Drinking Water Directive, which reviewed the quality standards and, where necessary, tightened them (adopted in November 1998) and a Directive for Integrated Pollution and Prevention Control, which addressed pollution from large industrial installations (adopted in 1996).

Rethinking the Community water policy started in the mid-nineties with special emphasis on the need for a more global approach to water policy. Whilst EU actions such as the Drinking Water Directive and the Urban Waste Water Treatment Directive can duly be considered milestones, European Water Policy had to address problems in a coherent way, *i.e.* not separated into topics such as drinking water and wastewater. This became the basis for a new European Water Policy, which was developed in an open consultation process involving all interested parties, *i.e.* local and regional authorities, water users and non-governmental organisations. The outcome of this consultation process was a widespread consensus that, while considerable progress had been made in tackling individual issues, the current water policy was fragmented, in terms both of objectives and of means. All parties agreed on the need for a single piece of framework legislation to resolve these problems. In response to this, the EC presented a Proposal for a Water Framework Directive with the following key aims:

- expanding the scope of water protection to all waters, surface waters and groundwater,
- achieving 'good status' for all waters by a set deadline,
- water management based on river basins,
- 'combined approach' of emission limit values and quality standards,
- getting the prices right,
- getting the citizen involved more closely and
- streamlining legislation.

In 1997 the EC proposed a European Parliament and Council Directive establishing a framework for Community action in the field of water policy, the Water Framework Directive or **WFD**. The Directive, which was adopted in September 2000 (2000/60/EC2), should "contribute to the progressive reduction of emissions of hazardous substances to water", the ultimate aim being "to achieve the elimination of priority hazardous substances (PHS) and contribute to achieving concentrations in the marine environment near background values for naturally occurring substances"[12]. In order to achieve this, pollution through the discharge, emission or loss of PHS must cease or be phased out. The WFD foresees that the development of water quality should be monitored by the member states on a systematic and comparable basis. Therefore, technical specifications should be laid down in order to assure a common approach, *e.g.* the standardisation of monitoring, sampling and methods of analysis. Although the WFD is designed for surface water and groundwaters, transitional (bodies of surface water in the vicinity of river mouths which are partly saline in character but mainly influenced by freshwater flows) and coastal (roughly the first mile of territorial waters) waters are also included. The WFD will therefore start playing a major role in the field of marine environmental monitoring and is very likely to put an additional burden on laboratories and scientists involved in this field. Although the Directive aims at making its contribution to earlier approved agreements such as OSPARCOM (*cf.* above), HELCOM and MEDPOL (see below), it imposes its proper demands for monitoring on member states.

The WFD identifies three types of monitoring – surveillance monitoring, operational monitoring and investigative monitoring. Surveillance monitoring provides information for assessment of the status of a river basin, and for the development of future monitoring programmes, and serves to monitor long-term changes under natural conditions and changes resulting from anthropogenic activity. In practical terms, surveillance monitoring is not continuous (intermittent periods), but is still very thorough. For the entire set of priority pollutants a monthly sampling scheme is foreseen for a period of one year. Operational monitoring is undertaken to assess the status of water bodies that are at risk of failing to meet the environmental objectives and to assess changes resulting from programmes of measures. Operational monitoring is continuous and follows the same frequency as surveillance monitoring. Although it is only intended for specific cases, it has severe implications both in time and effort. Finally, investigative monitoring is carried out if reasons for non-compliance with threshold levels are unknown, or

surveillance monitoring indicates that the objectives will not be met and operational monitoring is not yet established, or to investigate the impact of accidental pollution. Frequency and time of monitoring cannot be compared with both previous types, as this will be decided on a case-by-case basis.
The priority pollutants in the WFD and their selection procedure will be discussed in Section 1.3 below.

European Marine Strategy
On 2 October 2002, the EC published a Communication to the Council of the EU and the European Parliament entitled "Towards a strategy to protect and conserve the marine environment" (COM(2002) 539), which sets out objectives and related actions [19]. The Commission Communication represents the first step in the incremental development of the European Marine Strategy (**EMS**) for the protection and conservation of the marine environment.

The Commission's intention was to develop the EMS in close cooperation with member states, candidate countries, the European Parliament, European Economic Area (EEA) States (Norway and Iceland), the various, mainly regional, international organisations engaged in different sectoral aspects of the marine environment (such as OSPAR, ICES and IMO (International Maritime Organisation)), and with environmental non-governmental organisations and various sectoral industry associations. It is expected that Council conclusions will be reached in the very near future, thereby establishing the EU political framework for the further development of the EMS and the implementation – in coordination with the regional marine conventions such as OSPAR – of actions to achieve the objectives already identified in the Commission Communication of 2004 [20].

Coordination with existing programmes is thus an inherent part of the EMS. From the onset, it has been recognised that the Regional Marine Conventions/Commissions and Programmes, illustrated in Figure 1.2, play an important role at the interface between marine research and policy, both in the context of regional marine assessments and the development of measures for marine management. It has also been recognised that monitoring and assessment have a vital role when the ecosystem approach is applied to the management of human activities affecting the marine environment. Policy must initially be based on an assessment or evaluation of the state of the marine environment,

and the implementation of the latter must be followed by observation and assessment of what has, and has not, been achieved. Two working groups, the Working Group on Strategic Goals and Objectives (SGO) and the Working Group on European Marine Monitoring and Assessment (EMMA), have been created to develop the EMS. The SGO is, as its name suggests, identifying strategic objectives while EMMA has the task to develop practical solutions to the latter. On-going discussions in EMMA make clear that these regional assessments will play an important part in the context of pan-European assessments to be made under the framework of the EMS. Where there exist regional-seas monitoring and assessment programmes, these should be used as far as possible for new developments on EU and pan-European levels. Likewise, in developing existing EU measures – especially the EC Water Framework Directive – attention should be given to the links to both the pan-European and the regional-seas level [21,22].

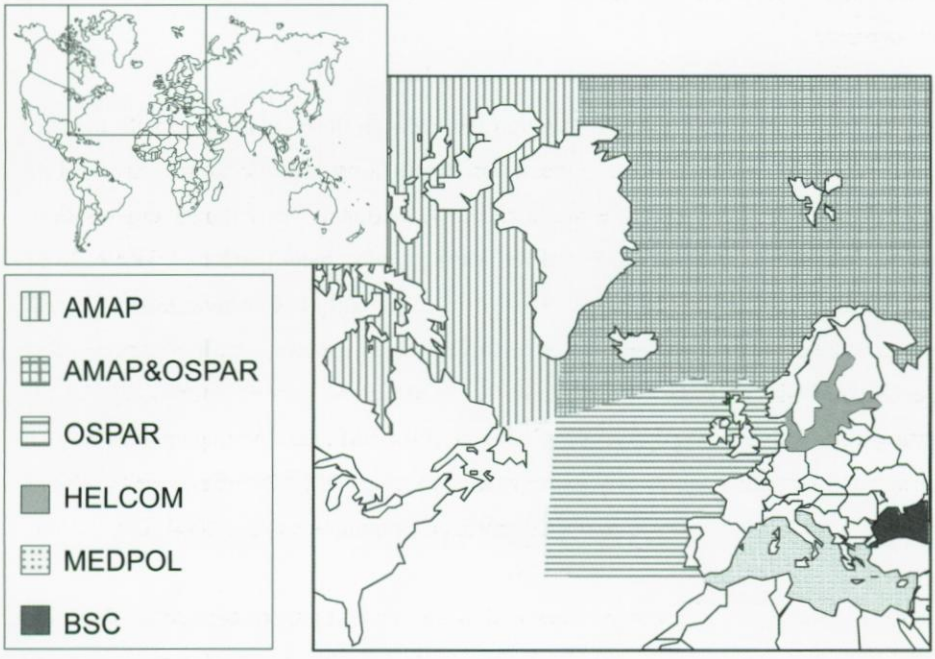


Figure 1.2: Regional Marine Conventions/Commissions and Programmes that are of relevance for the EMS. The programmes, given by their acronyms, are described in the text.

1.2.2. Other global and regional monitoring programmes

United Nations Environment Programme

Monitoring activities on a global scale are inevitably linked to the United Nations Environment Programme (**UNEP**). UNEP was established as a follow-up to the 1972 Stockholm Conference on the Human Environment, as the environmental conscience of the UN system [23]. With UNEP, a basis was created for comprehensive consideration and coordinated action within the UN on the problems of the human environment. UNEP particularly attempts to nurture partnerships with both other UN bodies and *e.g.* the scientific community and NGOs such as OSPAR.

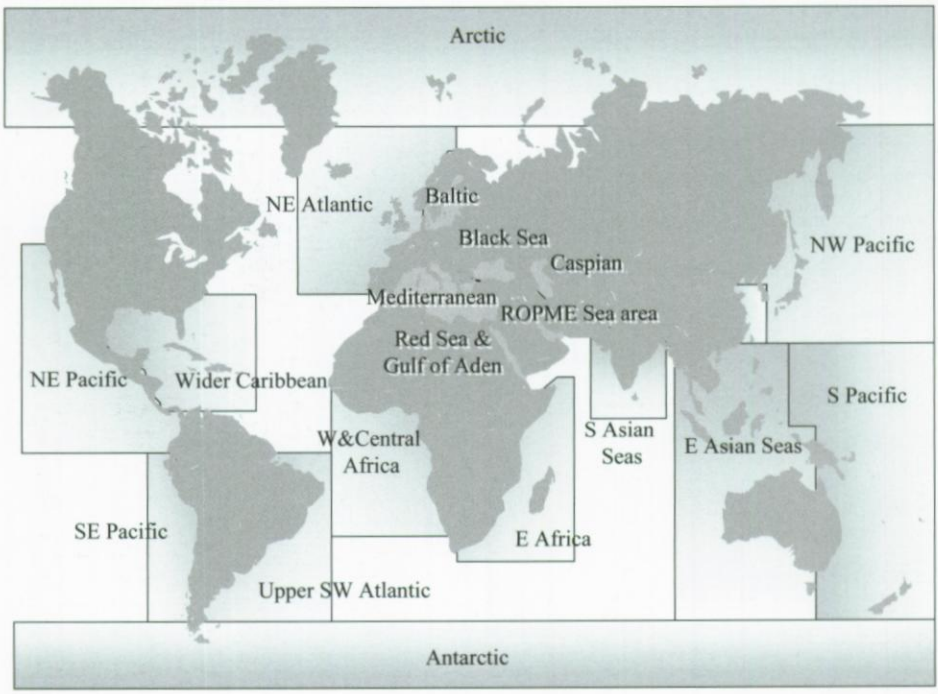


Figure 1.3: UNEP regional seas

UNEP has several water-related programmes. For instance, the Regional Seas Programme (**RSP**), initiated in 1974 as a global programme, includes 15 regions and more than 140 coastal states and territories (Figure 1.3). It is an action-oriented programme and focuses not only on the mitigation or elimination of the consequences, but also on the causes, of environmental degradation. The focus has gradually shifted from protecting the marine environment from pollution to striving at sustainable development of the coastal and

marine environment through integrated management. UNEP is also responsible for the secretariat set up to implement the 1995 Global Programme of Action (**GPA**) for the Protection of the Marine Environment from Land-based Activities. The UNEP Fresh Water Programmes comprise structured programmes of environmental inventory, analysis, diagnosis and action planning. Such programmes have been, or are presently being, developed and implemented for a number of large river and lake basins in Africa, Asia and South America.

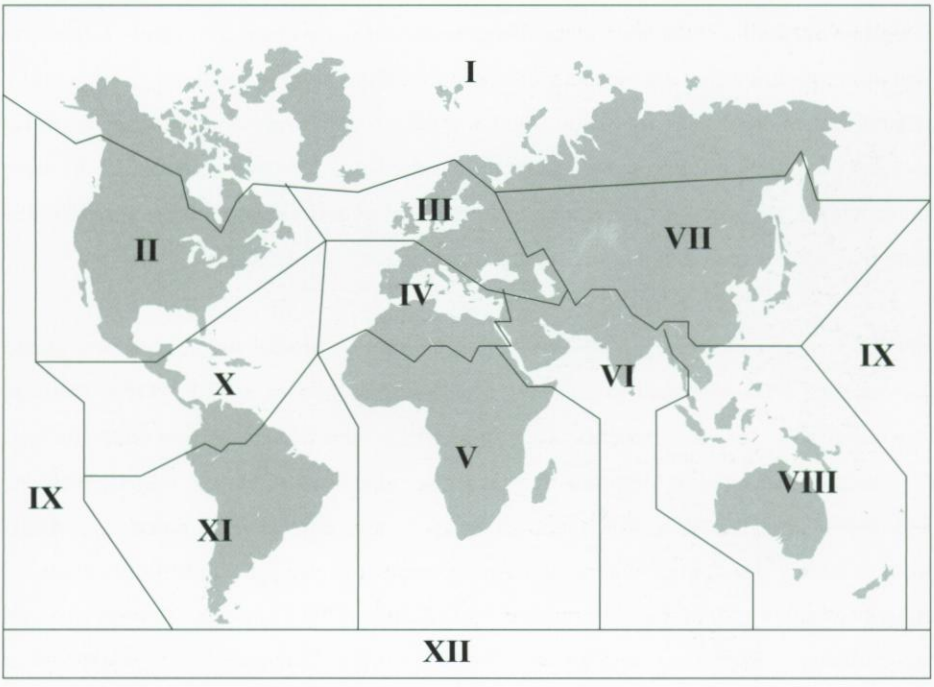


Figure 1.4: The twelve UNEP POP regions: the Arctic (I), North America (II), Europe (III), the Mediterranean (IV), Indian Ocean (VI), sub-Saharan Africa (V), central and northeast Asia (VII), southeast Asia and south Pacific (VIII), Pacific islands (IX), Central America and the Caribbean (X), eastern and western South America (XI), and Antarctica (XII).

UNEP is, furthermore, one of the implementing agencies for the Global Environment Facility (GEF). This is an independent international financing entity with the long-term goal to ensure progress toward global environmental security. The UNEP portfolio of GEF-funded activities in international waters includes global assessments, transboundary diagnostic analyses (TDAs) of shared water bodies, support for the implementation of strategic action programmes for marine and freshwater areas, and support for integrated management of shared freshwater bodies. Because water issues play an important and

increasing role in international development cooperation, GEF has designated international waters as one of its four focal areas.

The Global International Waters Assessment (**GIWA**), led by UNEP and 50% funded by GEF, will provide the information needed for GEF's work in this particular area. The aim of GIWA is to produce a comprehensive and integrated global assessment of international waters, the ecological status of – and the causes of environmental problems in – 66 water areas around the world, and to focus on the key issues and problems facing the aquatic environment in transboundary waters. The assessment is designed not merely to analyse the current problems but also to develop scenarios of the future conditions of the world's water resources and analyse policy options with a view to providing sound scientific advice to decision makers and managers concerned with water resources. In the near future, GIWA activities will be linked and coordinated with the monitoring programmes described elsewhere in this section, such as, *e.g.*, OSPAR and HELCOM.

The Stockholm Convention (2001) is a global treaty to protect human health and the environment from persistent organic pollutants (usually called **UNEP POPs**) that has been signed by 151 governments. In implementing the Convention, governments will have to take measures to eliminate or reduce the release of POPs into the environment [24]. POPs are chemicals which remain intact in the environment for long periods, become widely distributed geographically, accumulate in the (generally fatty) tissue of living organisms and are toxic to humans and wildlife. POPs circulate globally and can cause damage wherever they travel. The Stockholm Convention has identified a somewhat outdated list of twelve priority POPs, aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, PCBs, polychlorinated dibenzodioxins (PCDDs), PCDFs and toxaphene. The Stockholm Convention on POPs and other international agreements state that monitoring activities should be established to verify the effective implementation of the conventions and the decrease of environmental levels of persistent pollutants. Some monitoring activities are already in place but, as different methodologies are used, comparison of the data often is very difficult.

With support from GEF, UNEP has recently started a project called "Regional assessment of persistent, bioaccumulating and toxic substances or POPs" [26]. The project aims at collecting as much information as possible on the UNEP POPs for twelve regions in the

world (Figure 1.4), and, if possible, on more compounds than the 'twelve' quoted above. It is already clear that information is very sparse for several of the regions, and there is an obvious need to establish new monitoring activities. As with the OSPAR convention, the Stockholm Convention states that monitoring activities should be established to verify the effective implementation of the convention and the decrease of environmental levels of persistent pollutants. UNEP has therefore started another project, called "Global Network for the Monitoring of Chemicals in the Environment", which aims at linking national, regional and global organisations, laboratories and individuals responsible for, or involved in, monitoring of chemicals in man and the environment [27].

Other regional monitoring programmes

AMAP, the Arctic Monitoring and Assessment Programme, was established in 1991 to implement certain parts of the Arctic Environmental Protection Strategy (AEPS), primarily "providing reliable and sufficient information on the status of, and threats to, the Arctic environment, and providing scientific advice on actions to be taken in order to support Arctic governments in their efforts to take remedial and preventive actions relating to contaminants" [28]. The Arctic Council, established in 1996 by the eight Arctic countries (CA, DK, FI, IS, NO, RU, SE, and the US), coordinates AMAP activities. AMAP was conceived as a programme which integrates both monitoring and assessment activities in relation to pollution issues and provides information and reports on the state of the arctic environment. The AMAP Trends and Effects Monitoring Programme is designed to monitor the levels of pollutants and their effects in all compartments of the Arctic environment. There are five sub-programmes, which deal with atmospheric, terrestrial, freshwater and marine environments, and with human populations with respect to human health. The sub-programmes are defined in terms of essential and recommended parameters and media (matrices) to be monitored on a circumpolar or sub-regional level. The programme includes both monitoring and research components, and special studies that yield information which is vital for the valid interpretation of monitoring data. OMPs within the programme are PCBs, hexachlorobenzene (HCB), dioxins (PCDDs and PCDFs), pesticides (aldrin, chlordane, dieldrin, DDT, endrin, heptachlor, mirex, and toxaphene), PAHs, OTINs, short-chain chlorinated paraffins (SSCPs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBPA), perfluorooctanol sulphonic acid and its salts (PFOS) and polychlorinated naphthalenes (PCNs).

HELCOM, the Baltic Marine Environment Protection Commission or the Helsinki Commission, is the governing body of the Convention on the Protection of the Marine Environment of the Baltic Sea Area, signed in 1992 [29]. HELCOM's main goal is to protect the marine environment of the Baltic Sea from all sources of pollution, and to restore and safeguard its ecological balance. The present contracting parties to HELCOM are DE, DK, EE, EC, FI, LV, LT, PL, RU and SE. The set-up is very similar to that of OSPAR, and many of the principles – such as the 'best environmental practices', 'best available technologies' and 'the polluter pays' – are adopted and applied by HELCOM. Monitoring and assessment are an integral part of the convention and according to the convention "Emissions from both point sources and diffuse sources into water and the air should be measured and calculated in a scientifically appropriate manner by the Contracting Parties". Every five years, the Commission publishes a "Periodic Assessment of the State of the Environment of the Baltic Marine Area" based on monitoring activities going on in the area. The OMPs in the programme are virtually identical to those of AMAP and are mentioned in Table 1.1. Recently, a prioritisation of OMPs took place, taking recent developments and existing lists such as those of OSPAR, the WFD and UNEP POP into account [30]. The complete list is presented in Section 1.3 below.

MEDPOL, the Programme for the Assessment and Control of Pollution in the Mediterranean region was initiated in 1975 in Barcelona as the environmental assessment component of the Mediterranean Action Plan (MAP) and is now in Phase III [31]. Its task is to assist Mediterranean countries in the implementation of pollution-assessment programmes (marine pollution trend monitoring, compliance monitoring and biological effects monitoring). In parallel, MEDPOL provides assistance in the formulation and implementation of pollution control, regional and national action plans addressing pollution from land-based sources and activities. It also formulates and carries out capacity-building programmes related to the analysis of contaminants and treatment of data and to technical and management training. MEDPOL-collected data and information directly contribute to the implementation of the LBS (land based sources) and Dumping Protocols. The countries which signed the Barcelona Convention are AL, DZ, BA, HR, CY, EG, ES, FR, GR, IL, IT, LB, LY, MT, MC, MA, SI, SY, TN, TR and the EU. Currently, the targeted substances include PAHs, PCBs, HCB, TCDD, TCDF, OTINs, heavy metals and pesticides such as dieldrin, heptachlor, chlordane and mirex [32].

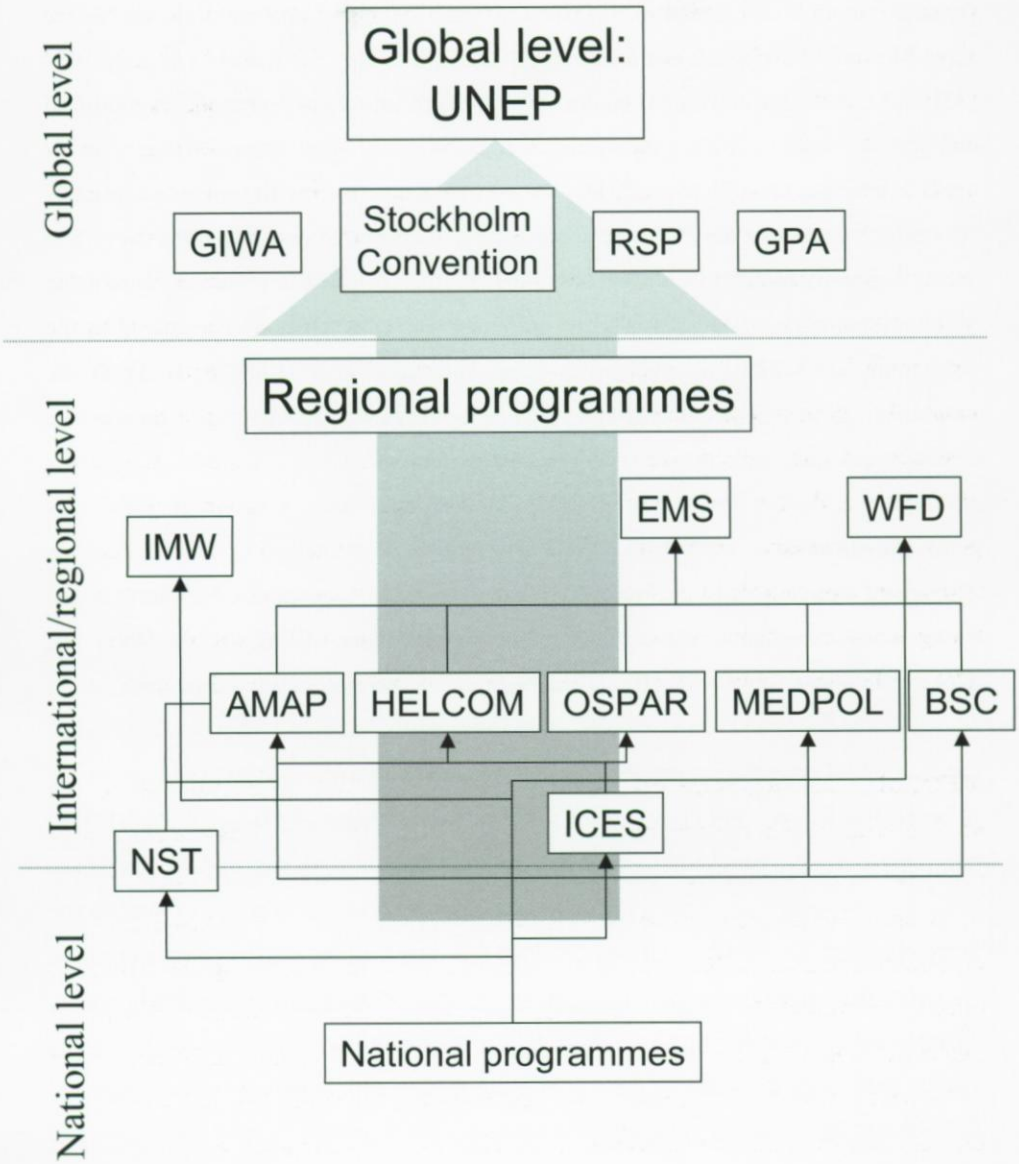


Figure 1.5: The idea of a global information flow in marine environmental monitoring. The grey arrow indicates the general direction of the flow. The black arrows indicate planned or present interactions between some important conventions and monitoring programmes. For abbreviations, see text

BSC, the Black Sea Commission or the Convention on the Protection of the Black Sea Against Pollution was signed in Bucharest in April 1992, and ratified by the legislative assemblies of all six Black Sea countries (BG, GE, RO, RU, TR and UA) in early 1994 [33]. The convention aims at (1) control of land-based sources of pollution, (2) control of dumping of waste and (3) establishing a framework for joint actions in the case of accidents such as oil spills. Specifically for the assessment and monitoring of pollutants a "State of Pollution of the Black Sea" report will be prepared and published every five years, beginning in 2006. It will be based on the data collected through the coordinated pollution monitoring and assessment programmes. The OMPs that are considered by the programme are OTINs, organohalogen compounds such as DDT, DDE, DDD, PCBs, persistent organo-P compounds and persistent substances with proven toxic carcinogenic, teratogenic or mutagenic properties.

1.2.3. Conclusions

The above overview is by no means complete but rather focuses on organisations and programmes immediately relevant for Europe as a whole and, specifically, the North Sea. There are various other regional and international monitoring programmes, such as the National Oceanic and Atmospheric Administration's National Status and Trends (NOAA's **NST**) monitoring programme that has been going on since 1986 and covers the Atlantic, Pacific and Gulf coasts of the US, and the International Mussel Watch (**IMW**) programme that covers Central and South America including Mexico and the Caribbean [34]. A global overview of the regional programmes, their common points of interest and interactions can be found in GESAMP (2001). In essence, the various programmes all recognise that organic micropollutants are an important threat to the marine environment, which should be carefully monitored, and, as a minimum, they share the following two goals: (1) to study the status of the marine environment with regard to contaminants and (2) to detect trends in concentrations. As a positive development, there is the tendency to make the various programmes mutually supportive rather than competitive. Figure 1.5 illustrates the current idea of information flow starting from national programmes, feeding into international/regional and, finally, global programmes. There is also a general awareness that the data which are produced, should be of high and, specifically, well-defined quality. The emphasis on quality assurance and quality control (QA/QC) is therefore omnipresent and has gained considerable importance in recent years. Amongst other things, this implies the obligation of laboratories to participate in

international intercalibration exercises and to use clearly described methodologies in the form of standard operating procedures [35].

1.3. Current state of marine environmental monitoring

The previous section shows that a number of monitoring programmes are currently active and/or have been so for many years or even decades. All early programmes deal with the industrialised northern hemisphere. The output of these programmes has been used to identify areas or regions of concern, estimate the hazards caused by OMPs to man and the marine environment and assess the effectivity of the measures taken.

Most organisations have published status reports that evaluate the quality of the, mostly regional, marine area they consider. This has provided a basic idea of the geographical distribution of OMPs in regions such as the Arctic [36], Baltic [37,38] and North Sea [10,13], and the Atlantic and Pacific coasts of North America [34,39]. These regional programmes also contributed significantly to obtaining an estimate of the global distribution of OMPs. The UNEP assessment of the worldwide presence of persistent toxic substances has, for the marine environment, greatly benefited from the information provided by these regional programmes [40]. In this report, sources, pathways and concentration ranges for the various compartments (water, sediment/soil, organisms) of both the terrestrial and the aquatic environment (marine and freshwater) are considered on a global scale. In general, the highest concentrations have been reported for DDTs, mostly DDE, PCBs (as the sum of individual congeners) and PAHs with concentrations that are up to 5-6 orders of magnitude higher than, *e.g.*, PCDDs/Fs (ng/kg concentrations). Most other OMPs such as HCB, lindane, PBDEs, toxaphene and modern pesticides, have concentration levels in between (µg/kg concentrations) [40]. As an illustration, the global concentration ranges of PCBs and DDTs in bivalves reported in the nineties are given in Figure 1.6.

The long-term monitoring programmes also demonstrate changes in the levels of OMPs in the marine environment. For instance, monitoring in the Baltic, which started in the late sixties, clearly showed the decline of several OMPs following actions to prevent their release [41,42]. This is illustrated in Figure 1.7, but note that concentrations seem to level out in the nineties. Decreasing PCB concentrations were cited in the 1993 North Sea

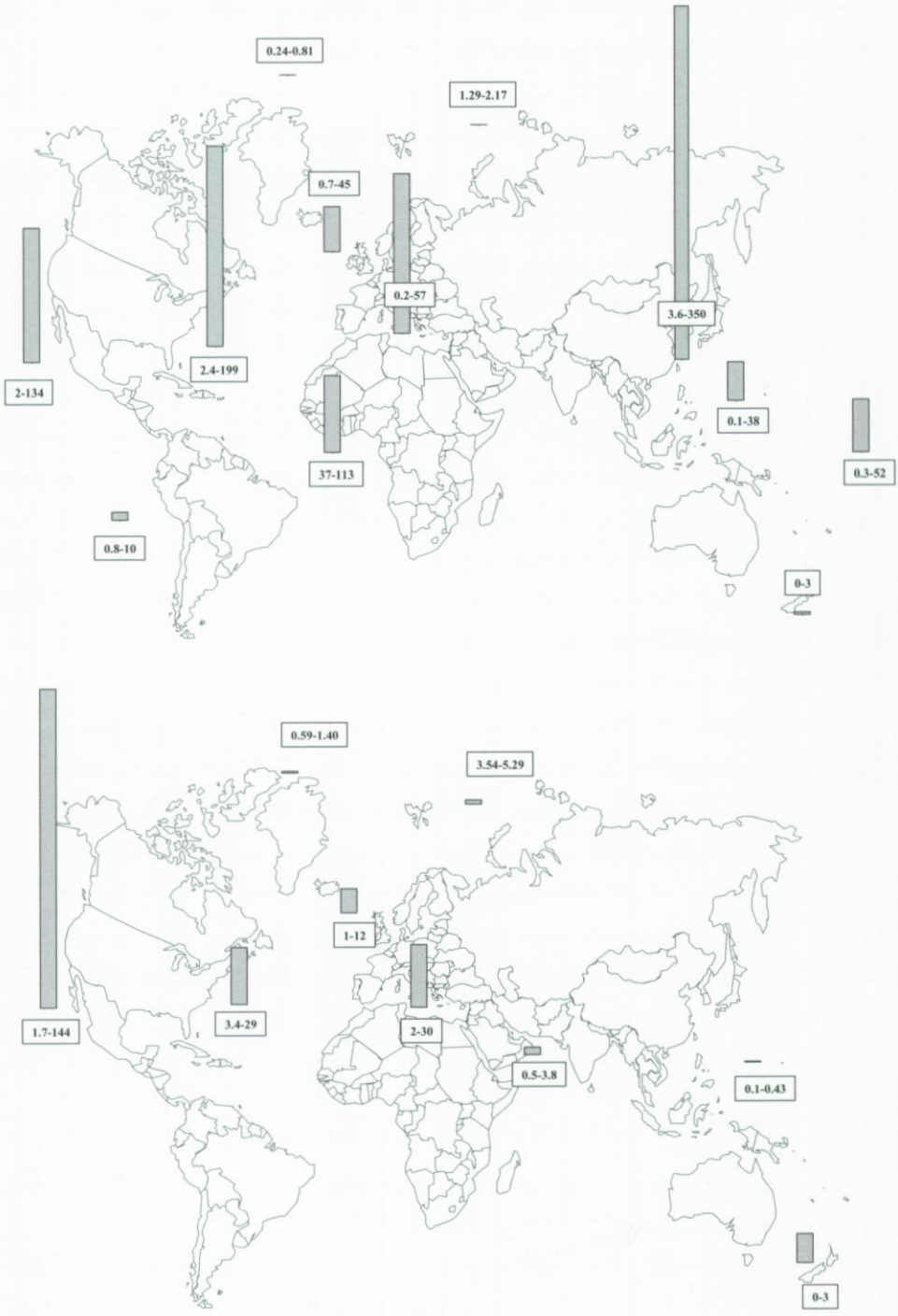


Figure 1.6: Concentration ranges of DDTs (top) and PCBs (bottom) in bivalves (µg/kg ww) reported during the 90s (source: UNEP Chemicals, 2003).

Quality Status Report for several species and various locations [13]. In the 1998 OSPAR assessment of trends of contaminants in organisms, significant downward trends were reported for HCB, γ-HCH, PAHs, CBs, DDTs and dieldrin [43]. Assessment of the data collected through NST and IMW [34] (see earlier) showed a general decrease in the total-DDT concentrations in molluscs for the northern part of the Gulf of Mexico, presumably as a result of the ban on DDT in the US in 1972. Decreasing concentrations were also found for PCBs, but not for PAHs. Brown *et al.* [44] found similar results for the Pacific coast of the US following a seven-year study during the National Benthic Surveillance Project of NST: PCBs and DDT concentrations were decreasing in fish and sediment, but PAHs showed no significant downward trend.

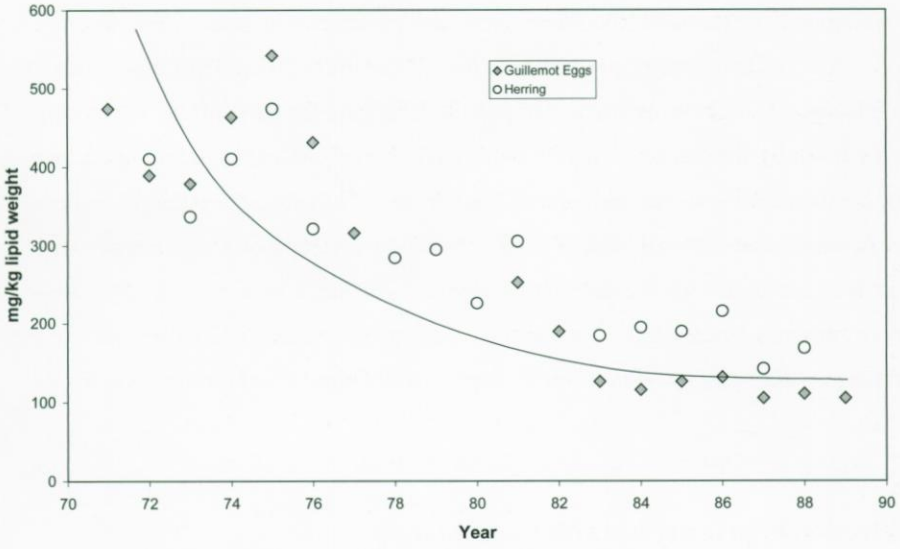


Figure 1.7: Decreasing trends of total PCBs (mg/kg lipid weight) in Baltic guillemot (eggs) and herring (after Bignert *et al.* [41]). Note that the absence of vertical bars indicating RSD ranges, hampers interpretation.

Despite the interesting results of the various programmes quoted above, some prudence is called for when interpreting the data. For example, in their status reports, most organisations tend to combine the contaminants in groups rather than to present the concentration of individual compounds. In the examples given above (Figure 1.6), the sets of target PCBs and the DDTs were not specified. Particularly for the PCBs it is well known that, on the one hand, the concentrations of the individual congeners are widely different while, on the other hand, the non-ortho and mono-ortho congeners are much

more toxic than all other congeners [45]. In addition, for most other classes of micropollutants, much less information is available which is, moreover, essentially limited to North America, the western half of Europe, Australia and Japan in virtually all instances. This problem was duly recognised in the 2003 UNEP assessment [40]. In other words, DDTs and PCBs are virtually the only pollutants for which it is possible to attempt making temporal and geographical comparisons [46]. This does not mean that there are no data available for other chemicals or for less developed parts of the world. However, in regions where surveillance networks are not operational, the information is generally the result of one-off surveys and more patchy. In addition, the information in the open literature is strongly targeted on the 'classical' pollutants such as PCBs, DDTs, PAHs, OCPs and PCDD/Fs. Fortunately, the threat posed by other contaminants is increasingly being recognised by the scientific community and governmental bodies. For instance, Muir *et al.* [47] recently noted that there is an overall lack of data on PTSs (Persistent and Toxic Substances) in North America. To quote an example, for most of the 100 priority chemicals listed by the Canadian government, there is very limited information on their current environmental levels, persistence and/or bioaccumulation. Actually, quite a number of them have not been studied at all. Obviously, selection of a significantly wide range of relevant chemicals for the marine environment has a high priority. This topic (which is receiving increasing attention in recent programmes and updates of older programmes) will be discussed in the next section, with emphasis on the situation for the North Sea.

1.4. Selection of priority hazardous substances

1.4.1. Introduction

Monitoring was initially limited to compounds entered on a 'red' list after a catastrophe or selected after their – sometimes accidental – detection in the environment (see above). In other words, in the absence of well-directed and/or targeted programmes, most chemicals were, and will be, 'missed'. On the other hand, it will be clear that it is impossible to determine concentrations of the approx. 250,000 man-made chemicals – the so-called chemical universe – in the marine environment [48]. Over the years this awareness led to the development of criteria that allow the prediction of which chemicals could be of concern for the marine environment. These selection criteria are briefly discussed below.

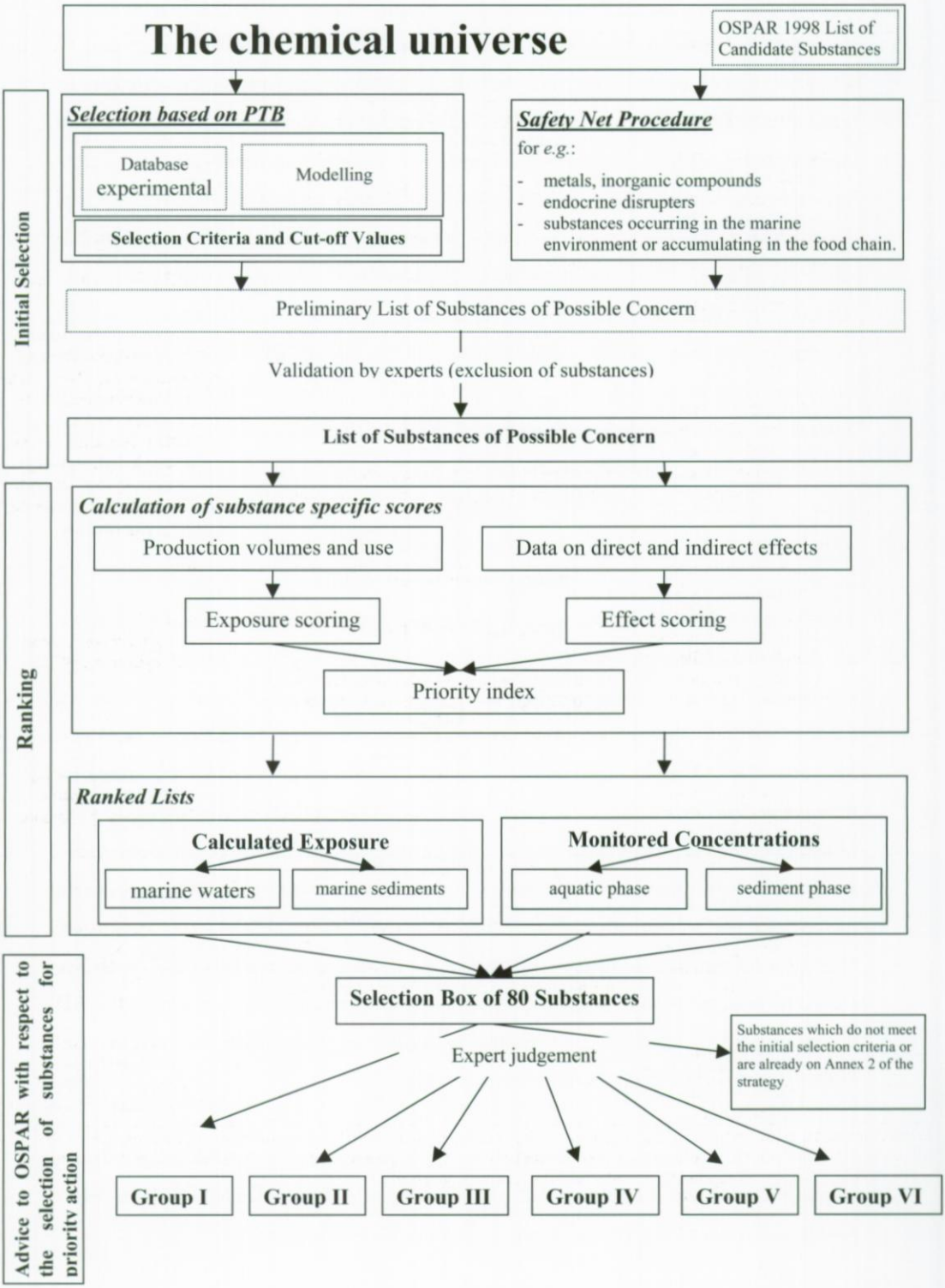
1.4.2. Selecting priority substances

In 1990, the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection and Advisory Committee on Protection of the Sea, GESAMP [48a], made a selection based on criteria such as the octanol/water partition coefficient or bioconcentration potential, acute toxicity, persistence, production volume and use of a chemical compound. The resulting list of potentially harmful substances contained mainly low-molecular-weight (MW) (C_1-C_3) chlorinated alkanes such as chlorinated methanes (*e.g.* dichloromethane, chloroform, tetrachloromethane), chlorinated ethanes (*e.g.* 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane), chlorinated ethenes (*e.g.* 1,1,2,2-tetrachloroethylene, 1,1,2-trichloroethylene), and medium-MW compounds such as chlorinated benzenes, phenols and toluenes, PCBs and PCDDs/Fs. In addition, an extensive list of compounds was identified for which insufficient data were available. In other words, next to the three most important characteristics, persistence, bioaccumulation and toxicity, the production volume and use were also taken into account. This resulted in the selection of chemicals such as dichloromethane. The same or similar approaches were also used in more recent assessments, as is exemplified below.

The threat of hazardous substances was recognised at the Third International Conference on the Protection of the North Sea (1990), which resulted in a list of 38 priority substances (Annex 1A) and an additional list of 170 potentially hazardous substances (Annex 1D) [49]. At the Fourth International Conference on the Protection of the North Sea (1995) or Esjberg Declaration (ED), the need for further development of criteria for defining and prioritising hazardous substances, which require action, was identified [50]. In addition, the need to develop risk assessment methods and measurement programmes for hazardous substances in the marine environment – specifically risk assessment methods for complex mixtures of substances and endocrine disrupters – was recognised. OSPAR's activities on hazardous substances are of special relevance to the North Sea Conference work, as the OSPAR Convention is an instrument to implement the ED targets. OSPAR's strategy with regard to hazardous substances was revised and implemented in 1998 in the 'Sintra statement' [51].

In response to its strategy concerning hazardous substances, the OSPAR Commission developed a dynamic selection and prioritisation mechanism, DYNAMEC, to select priority substances [52]. The entire process is illustrated in Scheme 1.

Scheme 1: The OSPAR DYNAMEC procedure.



Scheme 1: Contd.

Categories of priority hazardous substances and cut-off values for PTB criteria according to the OSPAR DYNAMEC procedure.

Group	**Description**	**Applied PTB cut-off values**	**Examples**
I	Substances of very high concern (*i.e.* POP-like substances or substances with PTB profile, selection I) and indication of production, use or occurrence in the environment	P: not inherently biodegradable *and* B: log $K_{ow} \geq 5$ or BCF ≥ 5000 *and* T_{aq}: acute $L(E)C_{50} \leq 0.1$ mg/l, long-term NOEC ≤ 0.01 mg/l *or* $T_{mammalian}$: CMR or chronic toxicity	2,4,6-*tris*(1,1-dimethylethyl)-phenol, dicofol, endosulphan, methoxychor, octylphenol, EPN, tetrasul, miconazole nitrate, diosgenin, trifluralin, clotrimazole
II	Other initially selected substances (with less severe PTB profile) and indication of use or exposure	P: not inherently biodegradable *and* B: log $K_{ow} \geq 5$ or BCF ≥ 5000 *and* T_{aq}: acute $L(E)C_{50} \leq 0.1$ mg/l, long-term NOEC ≤ 0.01 mg/l *or* $T_{mammalian}$: CMR or chronic toxicity	hexamethyldisiloxane, 1,2,3,4,5,5-hexachloro-1,3-cyclopentadiene, TBBA, 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, 1-(1,1-dimethylethyl)-4-methyl-benzene, cyclododecane, triphenylphosphine, isododecane, chlorpyrifos
III	Substances of very high concern (i.e. POP-like substances or substances with PTB profile, selection I) with no indication of use or exposure	P: not inherently biodegradable *and* B: log $K_{ow} \geq 5$ or BCF ≥ 5000 *and* T_{aq}: acute $L(E)C_{50} \leq 0.1$ mg/l, long-term NOEC ≤ 0.01 mg/l *or* $T_{mammalian}$: CMR or chronic toxicity	heptachloronorbornene, flucythrinate, PCNs
IV	Other initially selected substances with no indication of use or exposure		fenitrothion, isodrin, pentachloroanisole, fenpropimorph, diazinon
V	Substances with PTB properties but which are heavily regulated or withdrawn from the market	P: not readily biodegradable *and* B: log $K_{ow} \geq 5$ or BCF ≥ 5000 *and* T_{aq}: acute $L(E)C_{50} \leq 1$ mg/l, long-term NOEC ≤ 0.1 mg/l *or* $T_{mammalian}$: CMR or chronic toxicity	DDTs, chlordane, PCTs, aldrin, HCB, toxaphene , nitrofen, heptachlor
VI	Endocrine disrupters which do not meet P or B criteria or natural hormones		estradiol, estrone, diethylstilbestrol, 17-ethynylestradiol, butylphenol

P: persistence, B: bioaccumulation, T_{aq}: aquatic toxicity with $L(E)C_{50}$ the lethal (L) or effect (E) concentration that affects 50% of the population, NOEC: no observed effect concentration, $T_{mammalian}$: mammalian toxicity, BCF: bioconcentration factor; CMR: carcinogenicity, mutagenicity and adverse effects on reproduction.

Hazardous substances are defined as (groups of) compounds that are persistent, toxic and liable to bioaccumulate (PTB), or give rise to an equivalent level of concern through, *e.g.*, synergistic effects or degradation into hazardous substances [52].

During the initial selection, the following criteria were used:

- highly hazardous properties resulting in a general threat to the aquatic environment
- strong indications of risks for the marine environment
- widespread presence in one or more compartments of the marine environment
- potential threat to human health via consumption of seafood and
- presence of various pathways or a diversity of sources to the marine environment.

After the initial selection, a ranking based on a ranking algorithm was made. Final selection of substances for priority action was done by a group of experts. As with the GESAMP criteria quoted above, the selection heavily relies on the PTB criteria. However, in addition data on direct and indirect effects and production volumes and use are also taken into account. Calculated exposures and monitored concentrations were also considered. The initial selection led to a total of 80 substances or groups of substances, divided into five categories. Fifteen were selected as substances for priority action and another twelve as candidates for prioritisation [52]. The list of substances was updated in subsequent years. After the recent additions of PFOS, and the (pentabromo)methyl ester of 2-propenoic acid, the list now contains 47 chemicals for priority action [53]. The entire list of OSPAR's priority substances is given in Table 1.2. Fourteen of these have a lower priority although they have rankings in terms of persistency, bioaccumulation and toxicity that are of equal concern as for the other substances on the list. However, because they are used exclusively as an intermediate in closed systems in the production of other substances or because there is no current production or use in the OSPAR states, they have a lower priority (Table 1.2).

The OSPAR list played an important role during the selection of priority substances for the WFD. The basic procedure for the WFD was the Combined Monitoring-based and Modelling-based Priority Setting (COMMPS) procedure [54, 55]. Similar to OSPAR, hazardous substances means "substances or groups of substances that are toxic; persistent

and liable to bioaccumulate; and other substances or groups of substances that give rise to an equivalent level of concern". During the initial step of the COMMPS procedure, priority substances were again selected from among the list of hazardous substances, based on evidence regarding the intrinsic hazard, widespread environmental contamination and other proven factors which may indicate the possibility of widespread contamination such as production or use volume. Next, exposure indices – based both on surface water monitoring and on modelling data – and effect indices were calculated, which finally led to a risk-based priority index, essentially by multiplying both previous indices. This list was then submitted to expert judgement for the selection of priority substances. Interestingly, 'historic pollutants' such as PCBs were eliminated in this step [55]. Finally, the list was checked against the OSPAR list, other lists from EC regulations and lists resulting from international agreements, *e.g.* the Stockholm Convention on POPs [56]. The procedure resulted in a list of 33 priority hazardous (groups of) substances and an additional 10 priority (groups of) substances [57] (Table 1.2).

The OSPAR and EU approaches, which clearly show mutual influencing, provided the basis for priority setting under HELCOM [30]. As a result, a very similar list of 42 (groups of) substances was identified, which is included in Table 1.2. For further comparison, the Stockholm Convention or UNEP list of POPs, which contains 12 (groups of) chemicals, is also shown in Table 1.2.

1.4.3. Comparing the lists

Comparison of the lists discussed above clearly shows that there is much overlap, but that there are also several striking differences. Most surprisingly, not a single compound appears on all four lists. To facilitate further discussion, the priority substances of Table 1.2 have been sub-divided into several categories.

PCBs, OCPs (*e.g.* DDTs, aldrin, dieldrin) and PCDD/Fs are considered as the 'old' organochlorines and are also referred to as the 'old' contaminants [58]. The entire UNEP POP list consists of these substances, which makes it somewhat outdated; they are also priority substances for OSPAR and HELCOM. They have been the subject of extensive study and international regulation in recent decades. It is specifically because of the international attention that they are not on the WFD list (with the exception of hexachlorobenzene and HCHs). Although the initial COMMPS selection procedure

included most of them (even as top-ranking substances), they were not considered as priority substances because of the fact that there is no current production or usage, or use is strictly regulated or forbidden. Exclusion is therefore not based on toxicological properties and/or presence in the environment. In contrast, the latter is precisely the reason why organisations such as OSPAR and HELCOM consider them as priority substances, which seems a sounder approach.

The CBs are a particularly good example of this category. They have been a major cause for concern since their discovery in the environment by Jensen [3]. Large amounts of technical mixtures of CBs were manufactured by companies in the US, Japan and several European countries between 1930 and 1983 when their production – of, *e.g.*, approx. 36,000 tonnes in Europe alone in the period 1981-1984 – was discontinued in most countries [59]. During this period but, also, more recently, large quantities of CBs reached the environment through, *e.g.*, large-scale disposal, leakage, evaporation and accidents [60, 61]. Since the early eighties, CBs have been routinely monitored in a variety of marine samples, specifically, organisms and sediments. Most monitoring programmes require or suggest the analysis of individual congeners such as the 'ICES seven' (CBs 28, 52, 110, 118, 138, 153, 180). However, already in the 1980s it became clear that several CB congeners showed a dioxin-like toxicity [45], particularly non-ortho, but also mono-ortho CBs able to form a planar configuration and therefore bind to the Ah receptor in a way very similar to dioxins. Their toxicity is generally expressed by means of a toxic equivalent factor (TEF: ratio of toxicity of congener and 2,3,7,8-TCDD (tetrachlorodibenzodioxin)) [62]. Total toxicity is then calculated as Toxic Equivalent Quotients being $TEQ = \Sigma TEF_i \times c_i$, for all congeners, *i*, of interest. Although the concentrations of these CBs typically are 1000-fold less than those of the so-called indicator CBs, their toxicity is some 1000-fold higher [63]. Nevertheless, neither non-ortho nor mono-ortho CBs are part of most monitoring programmes and are, therefore, not routinely monitored, although their importance is recognised by, *e.g.*, WHO, OSPAR, HELCOM, QUASIMEME (Quality Assurance of Chemical and Biological Effects Measurements in MarineEnvironmental Monitoring) and the EU. One exception is the AMAP Trends and Effects Programme where, next to *e.g.* DDTs, planar CBs, are recognised as 'essential' in contrast to 'recommended' parameters for certain matrices such as sediment cores, fish liver and blubber of marine mammals [64].

Table 1.2: Overview of (groups of) substances selected by four international organisations

(Groups of) substances	OSPAR	WFD	HELCOM	UNEP POP
'Old' organochlorines				
Aldrin			X	X
Chlordane			X	X
DDTs			X	X
Dieldrin			X	X
Endosulphan	X	X[2]		
Endrin			X	X
Heptachlor			X	X
Hexabromobiphenyl			X	
Hexachlorobenzene		X[2]		X
Hexachlorocyclohexane isomers (HCH)	X	X[2]	X	
Isodrin	X[1]		X	
Mirex			X	X
PCBs	X		X	X
PCDDs	X		X	X
PCDFs	X		X	X
PCNs	X[1]			
Polychlorinated terphenyls			X	
Toxaphene (OSPAR: heptachlorononorbornene)	X[1]		X	X
'New' pesticides				
Acrylonitrile			X	
Alachlor		X	X	
Aramite			X	
Atrazine		X[2]		
Chlordecone			X	
Chlordimeform			X	
Chlorfenvinphos		X		
Chlorpyrifos		X[2]		
Dicofol	X			
Diuron		X[2]		
Ethyl *O*-(*p*-nitrophenyl) phenyl phosphonothionate (EPN)	X[1]			
Flucythrinate	X[1]			
Fluoroacetic acid and derivatives			X	
Isobenzane			X	

Table 1.2 contd.

(Groups of) substances	OSPAR	WFD	HELCOM	UNEP POP
Isoproturon		X[2]		
Kelevan			X	
Methoxychlor	X			
Morfamquat			X	
Nitrophen			X	
Pentachlorophenol (PCP)	X		X	
Quintozene			X	
Simazine		X[2]		
2,4,5-T			X	
Tetrasul	X[1]			
Trifluralin	X	X[2]		
VOCs				
1,2,3-Trichlorobenzene	X	X[2]		
1,2,4-Trichlorobenzene	X	X[2]		
1,2-Dibromomethane			X	
1,2-Dichloroethane		X		
1,3,5-Trichlorobenzene	X	X[2]		
Benzene		X		
Dichloromethane		X		
Trichloromethane		X	X	
PAHs				
Anthracene	X	X[2]		
Fluoranthene	X	X		
Naphthalene	X	X[2]		
Polyaromatic hydrocarbons	X	X[2]	X	
'New' organohalogens				
Brominated flame retardants (WFD: polybrominated biphenyls only)	X	X[2]		
1,3,5-tribromo-2-(2,3-dibromo-2-methylpropoxy)-benzene	X[1]			
Hexachlorobutadiene		X[2]		
Hexachlorocyclopentadiene (HCCP)	X			
Pentabromoethylbenzene	X[1]			
Pentachloroanisole	X[1]			

Table1. 2 contd.

(Groups of) substances	OSPAR	WFD	HELCOM	UNEP POP
Pentachlorobenzene		X[2]		
2-Propenoic acid, (pentabromo)methyl ester	X[1]			
Short-chain chlorinated paraffins (SCCPs)	X	X[2]	X	
Tetrabromobisphenol A (TBBP-A)	X			
Endocrine disruptors				
Nonylphenol/ethoxylates (NP/NPEOs) and related substances	X	X[2]	X	
Octylphenol	X	X[2]		
Phthalates: dibutylphthalate, diethylhexylphthalate (DEHP)	X	X[2]	X	
Other organic chemicals				
1,5,9-Cyclododecatriene	X[1]			
2,4,6-Tri-*tert*-butylphenol	X			
3,3'-(ureylenedimethylene)bis(3,5,5-trimethylcyclohexyl)-Diisocyanate	X[1]			
4-(dimethylbutylamino)-Diphenylamin (6PPD)	X			
4-*tert*-Butyltoluene	X			
Clotrimazole	X			
Cyclododecane	X[1]			
Diosgenin	X			
Hexamethyldisiloxane (HMDS)	X			
Musk xylene	X		X	
Neodecanoic acid, ethenyl ester	X			
Perfluorooctanol sulphonic acid and its salts (PFOS)	X			
Triphenyl phosphine	X			
Metals and related compounds				
Cadmium	X	X[2]	X	
Lead and organic lead compounds	X	X[2]	X	
Mercury and organic mercury compounds	X	X[2]	X	
Nickel and its compounds		X		
Organic tin compounds	X	X[2]	X	
Selenium and its compounds			X	

[1] Lower priority in OSPAR because of exclusive use as intermediates in closed systems or no current production and/or use in the OSPAR area; [2] first-priority hazardous substances.

The 'old' contaminants remain a cause of concern because of their persistence and because of continued releases and transport through the atmosphere of significant quantities of these chemicals or their transformation products, *e.g. p,p'*-DDE as a transformation product of *p,p'*-DDT [35,50,58]. There has long been concern over sublethal effects of long-term, low-level chemical exposure, particularly about the possibility of immunosuppression in mammals from both acute and chronic low-dose exposures [35]. Also, sublethal effects should be located at the level of critical biological processes such as reproduction, development and growth, which are mostly hormonally driven. Recent concern about endocrine-disrupting chemicals has led to significant new research on the hormonal effects of persistent (and some non-persistent) chemicals, amongst which are most of the twelve UNEP POPs [35]. Moreover and controversially, the fact that chemicals have already for a long time been recognised as important contaminants does not necessarily mean that their presence in the marine environment has been amply demonstrated. This is particularly true for the CDD/Fs. Quite recently, OSPAR's QSR highlighted the lack of data for these compounds in the marine environment [10], mainly due to costs involved in their ultra-trace level determination.

PAHs also belong to the group of 'old' contaminants. It is quite surprising that they are not on the UNEP-POP list, since they are recognised as priority hazardous substances by OSPAR, WFD and HELCOM. Also, Law [65] estimated that approx. 230,000 tonnes of PAHs reach the marine environment every year and are distributed worldwide. For the OSPAR area this is estimated to be around 8000 tonnes/year for the 'Borneff six' [66]. In the marine environment, PAHs tend to adsorb to particulate material as a result of their hydrophobic nature and to be deposited into the underlying sediments [67]. They are quite persistent, particularly in anaerobic sediments, and can accumulate to high concentrations [68]. The highest concentrations are generally found in coastal areas and in estuaries, with total PAH concentrations of up to 8.5 µg/l for the water phase and up to 6 mg/kg (dry weight) for sediments [66]. As a result of their high persistence and potential carcinogenic and mutagenic effects [69,70] PAHs should be considered as high-priority environmental pollutants [66]. In North Sea sediments the most carcinogenic PAH, benzo(a)pyrene, has been detected at concentrations up to 0.24 mg/kg [10]. PAHs have been routinely monitored since the 1970s. As is evident from Table 1.2, most major programmes identify specific individual PAHs and/or include them as a class. The most commonly used list of individual PAHs is that of the US EPA and contains 16 parent PAHs. However, OSPAR

has a list of only ten target PAHs while AMAP, on the other hand, has an additional 23 (groups of) alkylated PAHs next to the 16 EPA PAHs.

The other categories included in Table 1.2 – with the exception of metals, which are not considered here – can be considered as 'new' or emerging contaminants. A prominent group are the 'modern' pesticides. Again, there is much discrepancy between the lists and not a single pesticide is on all of them. The phenylurea pesticides diuron and isoproturon, the triazines atrazine and simazine and the anilide alachlor all belong to the group of semi-polar pesticides. With one exception, alachlor, they show up only on the WFD list. Much less polar and, typically fat-soluble pesticides include chlorpyrifos, chlorfenvinphos, trifluralin and dicofol. Their adverse PTB characteristics make them environmental hazards. Production volumes can be quite high: trifluralin, a dinitroaniline herbicide used to control a wide spectrum of annual grasses and broadleaf weeds in agriculture, has an annual production volume of 6,000 tonnes in the EU and a worldwide production of 24,000 tonnes [71]. They have been detected in concentration of up to 20-60 μg/l in seawater but little is known about their presence in sediments, fish and marine mammals. Most target pesticides are on the WFD list but, surprisingly, dicofol is only on the OSPAR list. This can be explained by the selection procedures. Although the initial selection was in all cases based on PTB (*cf.* above), the individual procedures tended to emphasize different criteria in their final selection. For instance, WFD applies a monitoring-based exposure scoring but relies exclusively on the freshwater aquatic environment for this – and mainly on the water column [55]. Many of the above compounds are indeed already monitored in the freshwater environment, hence their selection. This particular criterion is not applied by either OSPAR or HELCOM, which should explain the differences. Methoxychlor, which is only on the OSPAR list, is another good example. Although there is no hard evidence on current production and use in the OSPAR area, the pesticide is persistent, bioaccumulates and is very toxic to aquatic organisms. It also has potentially endocrine-disrupting properties. Because of this it was included by OSPAR in the List of Chemicals for Priority Action in 2000 [72].

Most of the pesticides mentioned above are well known and much research on them has been conducted in the field of freshwater studies. However, compounds such as acrylonitrile, aramite, isobenzane and kelevan (Figure 1.8) all taken from the HELCOM list, will appear somewhat exotic to most readers. Again, the selection procedure has to be

consulted. For its prioritization procedure, HELCOM self-evidently took the situation in the Baltic into account, which is not done by either DYNAMEC or COMMPS, which are not region-specific. In the Baltic, there are certain physical, chemical and biological features which (may) increase the vulnerability of this ecosystem to anthropogenic chemicals, which differ from the marine or freshwater environments addressed in the OSPAR and EU framework [30]. For instance, the Baltic is a semi-enclosed sea with a large catchment area, which will have obvious implications for, *e.g.*, trapping of chemicals, stocking up of chemicals in anoxic, deep sediments, the occurrence of stable hot spot sedimentation areas and a high input of hazardous substances. There are also socio-economic factors which may contribute to market occurrence and use of hazardous substances, that significantly differ from those on the EU market, *e.g.* the chemicals market and stocks of outdated hazardous chemicals in the Baltic countries, Poland and Russia. One example is the use of acrylonitrile in synthetic soil blocks as a grain fumigant and a pesticide; it was banned in Estonia only in 1999 [30]. The same is true for aramite, an extremely efficient miticide/acaricide used in the protection of fruits, vegetables and non-food plant crops from predation by mites (IRPTC). For the insecticides isobenzane and kelevan essentially no information is available concerning production, marketing and use – which illustrates a situation typical for the Baltic area.

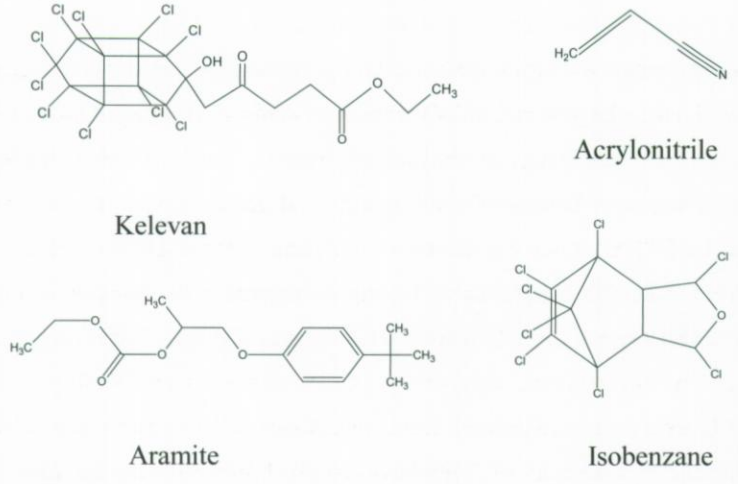


Figure 1.8: Structures of some pesticides that are of particular importance to the Baltic.

A good example of 'new' organohalogens are the polybrominated flame retardants (BFRs) which have received increasing interest from the community of environmental scientists in the last five years [73,74]. BFRs comprise compounds such as PDBEs, TBBP-A and brominated cycloaliphatic compounds such as HBCD. The annual world production of PBDEs has been estimated at 40,000 tonnes [75]. The use of PBDEs in the EU in 1994 was estimated at 11,000 tonnes [75] with a 75% contribution of deca-BDE. In 1999, over 9,200 tonnes of HBCD were used in the EU [75]. BFRs are persistent, bioaccumulate and are often transported over considerable distances. So far, most efforts in this field have been dedicated to the BDEs. Concentrations in the various compartments of the marine environment are, today, at least an order of magnitude lower than those of the CBs. However, most of the BDEs are still being produced and concentrations in the environment are reported to be increasing, *e.g.* in human milk, Beluga whales, guillemot eggs, marine mammals and sediments [75,76]. A recent survey of marine sediments in estuaries discharging into the North Sea showed deca-BDE concentrations from less than 0.5 to 1700 µg/kg dry wt. The highest level was detected in the Mersey estuary, at a site formerly used for dumping of sewage sludge and an area with much textile industry. The next highest level, 200 µg/kg, was detected in the Scheldt estuary [75], close to a production site. In marine mammals such as the sperm whale, BDE concentrations of about 100 µg/kg were found in blubber, which indicates that these compounds have reached deep ocean waters [73] and supports the hypothesis of long-range transport.

In this category, one should also mention SCCPs. They are formed by the chlorination of *n*-paraffins with chain lengths of 10-30 C-atoms and a chlorination degree of over 48 wt% [77,78]. SCCPs are very persistent and adsorb strongly to sludge and sediments and rapidly accumulate and biomagnify in aquatic food chains [79]. They are therefore included on the OSPAR List of Chemicals for Priority Action and are classed as PHS under the WFD. The 25th Adaptation to Technical Progress to the Dangerous Substances Directive 67/548/EEC has formally classified SCCPs as Category 3 carcinogens, and as dangerous for the environment. Releases of SCCPs to water from production or usage sites in the EU were estimated at some 1,800 tonnes/year, 95% being from metal-working sites [78]. In the past decades, SCCPs have mainly been produced for use in metal-working fluids (70%), but also as plasticisers (14%) and flame retardants (12%) [77,78]. In the middle nineties, the annual production in Europe was of the order of 15,000 tonnes,

but has been decreasing since then [78]. SCCPs have been found in a variety of water bodies and sediments, and also in fish and marine mammals [80-82]. However, generally speaking, there is a lack of environmental data on SCCPs which reflects the lack of sensitive, quantitative analytical procedures. Actually, most published methods are of a qualitative nature because even sophisticated capillary GC cannot create sufficient separation of the very many congeners. There also is a lack of suitable standards and, consequently, little information on relative response factors of individual congeners [82-84]. This problem will be further addressed in Section 1.4.

In view of the current concern about endocrine-disrupting chemicals, this category – which is recognised by all programmes except UNEP-POP – also deserves some attention. Although many of the 'old organochlorines' discussd earlier are also suspected to be endocrine disruptors, the alkylphenols have received specific attention in recent years. They are used to produce phenolic resins and alkylphenol polyethoxylates for use as surfactants in cleaners, wetting agents and emulsifiers [85]. Pentachlorophenol (PCP) is mainly used as a biocide in the wood industry. In the EU, production of PCP and its sodium salt was terminated in 1992. Today, these chemicals are imported from the USA. Production figures of octylphenol (OP), nonylphenol (NP) and nonylphenol ethoxylates (NPEOs) were some 25,000, 75,000 and 120,000 tonnes/year in the late 1990s [86,87]. Alkylphenols and short-chain alkylphenolethoxylates have fairly high log K_{ow} values of typically 4-6 and will therefore accumulate in sediment and, also, in marine biota [86-88]. OP was detected in water with concentrations of up to 13 µg/l in estuaries, but only up to 0.016 µg/l in coastal waters [86]. As for NPEOs, concentrations up to 9.5 mg/kg dry wt were found in the livers of river fish [87]. PCP has been detected in water, sediments and a large number of aquatic organisms. The Euro Chlor Risk Assessment for the Marine Environment (1999) showed that PCP concentrations are up to 0.8 µg/l for the North Sea, coastal waters and estuaries [89]. Recent literature shows that PCP concentrations in surface water and the marine environment of Western Europe are decreasing [90].

Although persistence and bioaccumulation are important factors in the selection process, these are not always decisive: Table 2 features many VOCs such as benzene, 1,2-dichloroethane, dichloromethane, trichlorobenzenes and trichloromethane. These well-known atmospheric contaminants are mostly important industrial compounds with a high annual production [91], which can be anywhere in the range from several hundred

thousand tonnes for *e.g.* tetrachloromethane, to more than 10 billion tonnes for benzene [92-94]. The low log K_{ow} values of the VOCs – typically 1-2 – led to the general belief that bioconcentration should be considered insignificant [93]. As a consequence, and also because of the considerable problems associated with the determination of VOCs, specifically in biota, there is a general lack of information. However, recent studies showed the general presence of several VOCs in the tissue of marine organisms from different levels of the food chain [95] with concentrations in marine organisms which are up to 1000-fold higher than in the surrounding water. The bioconcentration factors calculated from these data were generally higher than those reported in the literature, possibly due to the continuous exposure of the organisms to (very) low levels of these compounds in the water column. Determination in the water column alone is, therefore, insufficient.

Finally, amongst the group of miscellaneous organic chemicals, which was generated entirely by the OSPAR DYNAMEC procedure, several entries do not appear to be an obvious choice. Discussion of a few examples seems to be of interest. Clotrimazole is a pharmaceutical that is mainly used for treatment of dermatological and gynaecological fungal infections. It was selected by the OSPAR procedure on the basis of its resistance to biodegradation and its toxicity [96]. Hexamethyldisiloxane (HMDS) is used as an intermediate in the synthesis of *e.g.* polymers and as an ingredient in personal-care products and solvents. In 2000, the total production of HMDS in the EU was 4025 tonnes [97]. The rather high production, and also the persistence of HMDS probably effected its inclusion in the OSPAR list. A recent assessment of the environmental risk of HMDS concluded that it poses no risk to the marine environment on the regional scale, but that there is a potential risk on the local scale [97]. Finally, 4-*tert*-butyltoluene is used primarily as a raw material in the production of *p*-*tert*-butylbenzoic acid, which is utilised in the manufacture of unsaturated polyesters and alkyd resins [98]. The compound was selected on the basis of its ability to bioaccumulate and its persistence, although it shows a low acute aquatic toxicity [98].

Table 1.3: List of OMPs expected to remain and/or become priority target compounds for the North Sea area.

(Groups of) substances	Preferred matrices	Analytical methods available*
'Old' organochlorines		
Aldrin	sediment, biota	GC-MS
Chlordane	sediment, biota	GC-MS
DDTs	sediment, biota	GC-MS
Dieldrin	sediment, biota	GC-MS
Endosulphan	sediment, biota	GC-MS, LC-MS
Endrin	sediment, biota	GC-MS
Heptachlor	sediment, biota	GC-MS
Hexachlorobenzene	sediment, biota	GC-MS
HCHs (*e.g.* lindane)	water, sediment, biota	GC-MS
Metoxychlor	water, sediment, biota	GC-MS, LC-MS
Mirex	sediment, biota	GC-MS
PCBs	sediment, biota	GC-MS, GC-ECD
PCDDs	sediment, biota	GC-MS, GC-ECD
PCDFs	sediment, biota	GC-MS, GC-ECD
PCNs	sediment, biota	GC-MS, GC-ECD
Toxaphene	sediment, biota	GC-MS**
'New' pesticides		
Alachlor	water, sediment, biota	GC-MS, LC-MS
Atrazine	water, sediment	GC-MS, LC-MS
Chlorfenvinphos	water, sediment	GC-MS, LC-MS
Chlorpyrifos	water, sediment	GC-MS, LC-MS
Dicofol	water, sediment	GC-MS, LC-MS
Diuron	water, sediment	LC-MS
Isoproturon	water, sediment	LC-MS
Simazine	water, sediment	GC-MS, LC-MS
Trifluralin	water, sediment	GC-MS, LC-MS
VOCs		
1,2,3-Trichlorobenzene	water, sediment, biota	GC-MS
1,2,4-Trichlorobenzene	water, sediment, biota	GC-MS

Table 1.3 contd.

(Groups of) substances	Preferred matrices	Analytical methods available
1,2-Dichloroethane	water, sediment, biota	GC-MS
1,3,5-Trichlorobenzene	water, sediment, biota	GC-MS
Benzene	water, sediment, biota	GC-MS
Dichloromethane	water, sediment, biota	GC-MS
Trichloromethane	water, sediment, biota	GC-MS
PAHs		
Priority set and/or individual PAHs	sediment, biota	GC-MS, LC-FLU or LC-MS
'New' organohalogens		
BFRs: PBDEs, HBCD, TBBP-A	sediment, biota	GC-MS/LC-MS***
Hexachlorobutadiene	sediment, biota	GC-MS
Pentachlorobenzene	sediment, biota	GC-MS
SCCPs	sediment, biota	GC-MS **
Endocrine disruptors		
NP/NPEOs and related substances	water, sediment, biota	LC-MS
Octylphenol	water, sediment, biota	LC-MS
Dibutyl and diethylhexyl phthalate	water, sediment, biota	GC-MS***
PFOS	water, sediment, biota	LC-MS

* 'MS' comprises all current techniques, primarily quadrupole, triple quad, ion-trap and time-of-flight MS; ** adequate analysis is not yet possible; *** analysis has been described but there are still problems.

1.5. Monitoring: conclusions and trends

1.5.1. Future monitoring in the North Sea area

Monitoring in the North Sea has been already for a long time, and still is greatly influenced by OSPAR. This could easily lead to the conclusion that the OSPAR list mentioned above will constitute the list of compounds for future monitoring in this ecosystem. However, as the WFD list is legally binding for European Union countries, many of which are contracting parties to OSPAR, and as the European Marine Strategy (EMS) is, in a way, potentially extending the WFD into the open sea [22], the list proposed by the WFD will, in our opinion, be the main driving force for future

monitoring in the North Sea. Actually, OSPAR has already studied the compatibility of its own monitoring programme and that of the WFD. It also has recently decided that it would like to let the EU take the lead in future decisions on prioritisation of hazardous substances [22]. Furthermore, the Stockholm UNEP-POP Convention is a global convention and commitments made in this framework supersede all regional obligations. This means that the agreed, and admittedly, list of twelve POPs (*cf.* Table 1.2) should become part of all national and regional surveys – or that, at the very least, information should be gathered concerning their presence in the environment.

In Table 1.3 we have tried to summarise the above by indicating which OMPs will probably be important for future monitoring of the North Sea area. Our selection is primarily based on the WFD list but in contrast to the conclusions of the WFD, some additional OSPAR priority substances and the 'old' organochlorines are still included in our list, partly because they are on the UNEP-POP list but also because of their environmental relevance. For most groups of compounds in this category, it still has to be decided which congeners or isomers will have to be monitored. Although several lists of congeners are currently accepted, they may need to be revised. For instance, we have already mentioned that the selection of CB congeners is limited to a number of key CBs such as the 'ICES seven', while the highly toxic planar CBs (non-ortho an mono-ortho CBs) are not included in most instances (*cf.* Section 1.4). It seems recommendable that these congeners should become part of future monitoring activities as is already the case for the Arctic Monitoring and Assessment Programme (AMAP; see Section 1.2). A similar decision should be taken for the CCD/Fs – with the seventeen 2,3,7,8-substituted congeners being an obvious choice – and the PAHs, where the sixteen EPA priority PAHs are a good candidate set. More problems will no doubt be encountered with the PCNs and PBDEs. Analysis of these less exhaustively studied compound classes does not appear to be particularly difficult, but, until very recently, a lack of standards of the individual congeners and, more importantly, the paucity of PTB data prevents making a well-founded choice. Toxaphene and the SCCPs – both comprising many thousands of congeners and isomers – require still more attention. In this case, adequate separation has, so far, met with dramatic problems (see Section 1.5.2).

As a consequence, the synthesis of properly selected congeners and an in-depth study of PTB characteristics is still in its infancy.

From Table 1.1 in Section 1.1 and the discussion on monitoring programmes in Section 1.2 it is clear that a substantial number of the (groups of) compounds included in Table 1.3 are currently not being monitored routinely in the marine environment. However, this should not be taken to imply that suitable analytical methods have not been reported and/or that no survey-type information on these compounds is available at all. As for the former aspect, which will be discussed in some more detail in Section 1.5.2, the right-hand side column of Table 1.3 clearly indicates that the number of problems is limited. As regards the latter topic, there have been several promising, but rather short-term studies conducted by specialized laboratories, for example on musk xylenes [99,100], on VOCs [95,101,102] and also on emerging pollutants such as tri-(4-chlorophenyl)-methanol and tri-(4-chlorophenyl)-methane in flatfish [103]. The results of such a survey on VOCs in eel [101] has prompted the Flemish government to consider inclusion of these compounds in its eel-monitoring programme next to heavy metals and CBs.

As for the list of matrices included in Table 1.3, our preference is based on both experimental evidence taken form the literature and, more simply, the generally observed accumulation of hydrophobic compounds in sediment (and biota). Here it is also of some interest to note that the WFD is currently in the process of proposing the water column as the most appropriate matrix for its list. This is mainly because environmental quality criteria (based on toxicological tests) are mostly available for the water phase and cannot be converted directly to sediment or biota. However, to our opinion this is not a fortunate choice in view of the many apolar compounds listed in Table 1.3. Marine scientists have amply demonstrated that sediments and biota are much more suitable sample types for such analytes, while water is a proper matrix for more polar compounds such as, *e.g.*, the 'new' pesticides, which are indeed frequently detected in coastal and estuarine waters.

1.5.2. Analytical procedures

The analytical strategy generally used to arrive at the desired outcome, the concentration data of the target analytes, is briefly sketched in Figure 1.9. The sampling process itself is not included in the flow chart. Suffices it to say that sampling at sea is a very expensive

and labour-intensive affair with, frequently, a substantial reduction of the number of samples actually collected compared with that initially intended, owing to bad-weather conditions in the sampling area and/or technical difficulties during the campaign. To quote an example, an estimated 9% of the intended data were not received for the Belgian sediment monitoring programme in the North Sea in the period 1990-2003, because of the conditions at sea, but virtually none due to errors made during the actual analysis in the laboratory.

Sample preparation. As far as water samples are considered – and, consequently, the more polar analytes of Table 1.3 such as the modern pesticides and the NP/NPEOs – liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are essentially the only techniques in use. Rather large sample volumes of, typically, 5-20 l are collected and analyte extraction is done on board the ship to minimize analyte degradation and to facilitate transport. Detailed information on a few selected procedures is presented in Table 1.4, taken from an extensive recent study by Steen [104]. One important aspect is that on-line filtration and extraction of the target analytes from river and estuarine samples with high suspended-particulate-matter concentrations and DOC contents may well take ten times as long as from relatively clear marine waters. This should be kept in mind especially when consulting one or more recent reviews on sample preparation of aqueous samples for subsequent analysis by GC-MS (off-line and on-line) or LC-MS (usually on-line): their focus generally is surface and groundwater rather than water from estuaries or the open sea [113-116]. One should also consider that the limits of detection (LODs) that have to be achieved for the marine environment, are much lower – *i.e.* 1-5 ng/l – than the, typically about 100 ng/l, values quoted in such reviews.

The analysis of biota and sediments requires the use of extraction techniques which allow the release of the analytes from the matrix. Soxhlet extraction – inclusive of such modifications as hot Soxhlet extraction, the use of binary non-polar/polar solvent mixtures and semi-automated Soxtec – is still the benchmark and is most commonly used for the extraction of virtually all (classes of) analytes in Table 1.3, even for emerging contaminants such as the PBDEs [63,116,117]. However, there have been numerous attempts to find alternative procedures which are less time-consuming, use less solvent and/or enable miniaturisation. Amongst these novel approaches are pressurized liquid extraction (PLE) and related subcritical water extraction (SWE), microwave-assisted

extraction (MAE), matrix solid-phase dispersion (MSPD) and ultrasound extraction (US). Supercritical fluid extraction (SFE), an older and frequently less appreciated technique, should be added to this list because of its fat-removal potential.

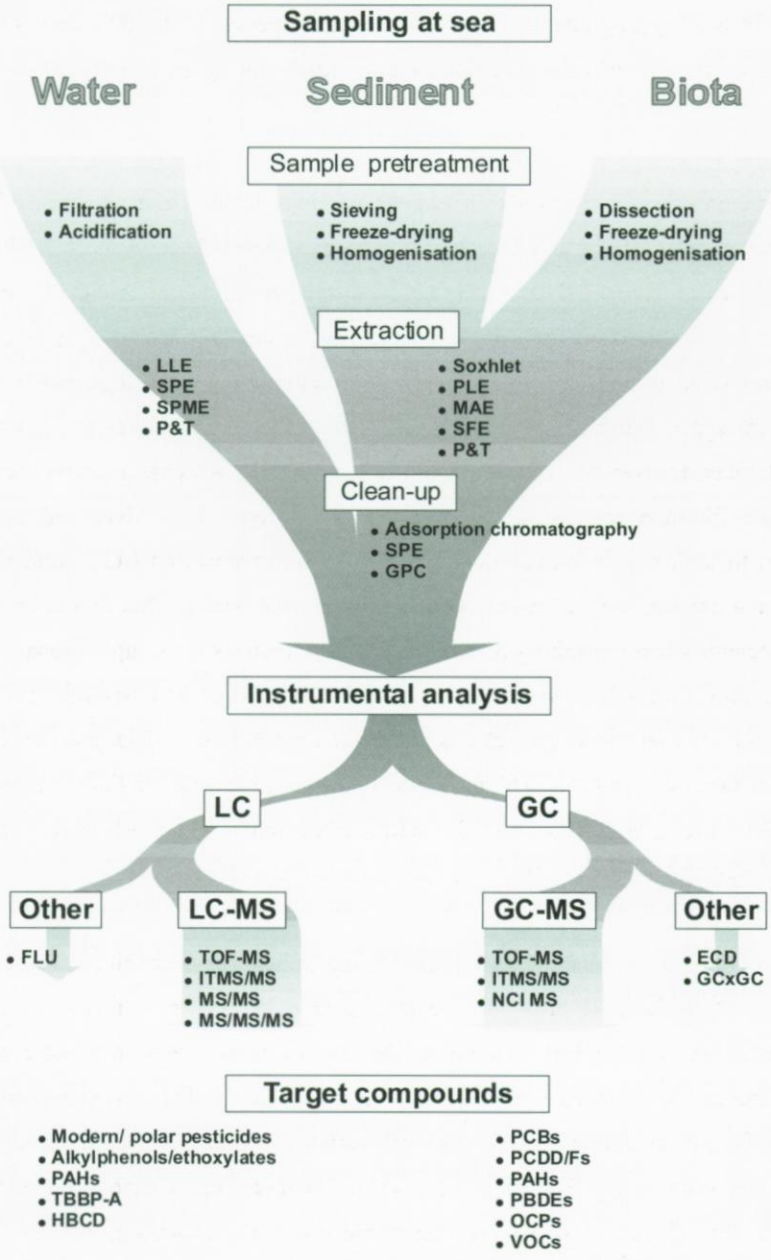


Figure 1.9: Analyte strategies for the determination of organic micro-contaminants in the main marine environmental matrices, water, sediment and biota.

Table 1.4: Examples of large volume extraction methods for the determination of pesticides in river and marine waters from Steen [104].

Compounds	Water sample	Sample volume (l)	Extraction technique, solvent and/or sorbent	Analysis	LOD (ng/l)	Ref.
Acidic compounds	river	20	GLSE[a]; DCM	GC-MSD	1-10	[105]
Model substances including pesticides	sea	10	SPE; 2 g SDB-1[b]	GC-ITDMS	0.1-0.7	[106]
Triazines, OPPs[c], acetanilides	river/estuarine	10	SPE; stacked cartridges 2-4 g GCB[d] or 10 g C18	GC-MS	0.5-3	[107]
Triazines, OPPs, acetanilides, OCPs	marsh	10	SPE; 90 mm C18 disks	GC-MS	0.05-2	[108]
Triazines	sea	5	SPE; 47 mm C18 disks	GC-NPD or GC-MS (SIM)	0.02	[109]
Triazines	river/estuarine	5	LLE; DCM	GC-MS	0.1	[110]
Acidic herbicides, bentazone	estuarine	5	SPE; 47 mm SDB disks	LC-DAD UV	50-100	[111]
Triazines, OPPs, acetanilides, TPs[e]	river and simulated sea	4	SPE; 47 mm C18 disks	LC-DAD UV	10-20	[112]

[a]GLSE = Goulden large-sample extractor; [b]on-line filtration-extraction with styrene-divinylbenzene copolymer (SDB); [c]OPPs = organophosphorus pesticides; [d]GCB = graphitised black carbon; [e]TPs = transformation products.

Due to the low selectivity of most of the quoted techniques, the crude extracts usually contain a large number of interfering compounds – frequently in relatively large amounts – and further clean-up and/or fractionation is required. Conventional and well documented procedures are solid-liquid adsorption in open columns packed with, *e.g.*, Florisil, silica or alumina and off-line or on-line SPE on a variety of bonded silicas or gel permeation chromatography. Specifically when ultra-trace analysis at the low ng/kg level has to be performed – as, *e.g.*, in the case of planar CBs and the priority CDD/Fs – carefully optimised multistep procedures have to be applied [63,117-120].

To illustrate the general interest in introducing the novel techniques, a series of selected applications is summarised in Table 1.5. There is, to our opinion, little doubt that the modern extraction techniques will at least partly replace the traditional methods, because they enable some of the demands mentioned above, to be fulfilled. However, a variety of techniques and different sets of optimum conditions will always be needed, because of the highly divergent analyte/matrix combinations that have to be considered. Consequently,

there is little need to discuss the quoted, or other similar, examples in detail: it is the general trend in sample preparation that requires our attention. Even so, it is worthwhile to stress a few aspects of interest.

From among the techniques mentioned, PLE has – so far – been most successful, even though care has to be taken that no decomposition of thermolabile compounds occurs during extraction. Interesting modifications include in-cell clean-up of samples by blending them with, *e.g.*, Florisil, alumina or a chemical used for extract purification in classical procedures [121], and the use of a small carbon column in the extraction cell, which selectively adsorbs dioxin-like compounds (subsequently isolated by back-flushing with toluene) [122]. PLE and MAE have the shared advantage over SFE that they are matrix-independent, which facilitates method development. SWE essentially is a PLE-type procedure with water as the extraction solvent. In contrast to PLE, pressure has no effect and only the temperature has to be optimised. Admittedly, SWE causes extracts to be rather diluted but, as water is the solvent, (on-line) combination with SPE, LC and/or SPME opens interesting perspectives [121]. Recent years have seen an increased use of ultrasound-based techniques for analyte isolation from solid samples. With most applications, extraction efficiency is fully satisfactory, and sonication time often is 30 min or less. Volatile and/or thermolabile compounds can be handled since high temperatures are not used [123].

As far as sample preparation is concerned, VOCs are the only group of compounds in Table 1.3 that require a totally different approach. For these compounds extraction is invariably based on their high volatility and is, therefore, matrix independent. Dynamic headspace techniques, usually called purge-and-trap (P&T), have emerged as the leading technique for marine samples [102]. Other techniques such as static headspace, solid-phase microextraction (SPME) and membrane inlet mass spectrometry (MIMS) can also be applied, but because of the low VOC concentrations (ng/kg range) in marine samples, LODs obtained when using these methods are generally not satisfactory. P&T has proven to be an exhaustive and reliable technique. Unfortunately, it is also labour-intensive and requires rather complex instrumentation [95,102,151]. However, if sample volumes can be kept small (<200 ml), partial automation of the procedure is possible. No clean-up is required and the P&T device can be coupled directly to the GC-MS.

Table 1.5: Selected applications of novel extraction procedures for OMPs in solid matrices.*, **

Compounds	Matrix	Pre-treatment	Clean-up	Analysis	Ref.
PLE [121,124, 125,126]					
PAHs	mussel	freeze-dry	GPC	GC-MS	[127]
PAHs	sediment	air dry, sieve		GC-MS	[128]
4-NP	sediment	freeze-dry, grind, sieve	Al_2O_3	LVI-GC-MS	[129]
PCBs, OCPs	fish (CRM)	Na_2SO_4	GPC	GC-MS	[127]
SCCPs	fish muscle	Na_2SO_4	GPC, Florisil	GC-ECNI-MS	[130]
NP/NPEOs	marine sediment		SPE	LC-ESI-MS	[131]
PFOS	sewage sludge	freeze-dry	oxidative digestion, SPE	LC-MS and -MS/MS	[132]
SWE [121]					
PAHs	marine sediment	dry, homogenise	In-cell SPE	GC-MS	[133]
PCBs	sediment	dry, sieve	SPME	GC-ECD	[134]
BFRs	sediment	dry, homogenize, sieve	On line Tenax	LVI-GC-ECD	[135]
MAE [136]					
PBDE	marine mammals		GPC, SiO_2	GC-MS	[137]
PCBs, HCHs, DDTs, toxaphene	fish tissue	freeze-dry	GPC	GC-MS	[138]
Trichlorobenzenes	fish tissue	homogenise	Al_2O_3, SiO_2, Na_2SO_4	GC-ECD	[139]
US [123]					
Phthalates	river sediment			GC-MS	[140]
NP/NPEOs	estuarine sediment	drying	RP-LC	LC-ESI-MS	[141]
PAHs	sewage sludge				[142]
SFE [124,143]					
PBDE	sediment	drying, mixing and Cu		GC-MS	[144]
PCBs	harbour sediment (CRM)	Na_2SO_4 and Cu		GC-MS	[145]
PCBs, DDTs	fish muscle	freeze-dry		GC-MS	[146]
NP/NPEO	mussels, sediment		SiO_2	GC-MS	[147]
MSPD* [148]***					
pharmaceuticals,	river sediment		SPE	LC-ESI-and APCI-MS/MS	[149]
NP/NPEOs	fish tissue	homogenise		LC-FLU	[150]

* Step in-between pre-treatment and clean-up; ** For recent reviews, one is referred to the references added to the sub-headings; ***MSPD = matrix solid-phase dispersion.

The only serious interference is caused by the considerable amounts of water vapour generated during purging. This is particularly so for solid samples such as biota and sediment, where elevated temperatures (of up to 70°C) are required to force the VOCs out of the sample. On-line water removal via a cold trap then has to be included. Under these conditions, foaming is regularly observed for biota samples. This drawback can, however, easily be solved by adding some *n*-octanol to the sample [152].

Instrumental analysis. Although it may come as something of a surprise, there is little doubt that, for priority hazardous substances such as are considered in Tables 1.2 and 1.3, GC-based analytical procedures will remain predominant in years to come. After all, most priority compounds have been selected on the basis of their PTB properties and are low-polarity compounds which are ideally suited for analysis by means of GC-MS. One example was already mentioned above, *viz.* on-line and automated P&T-GC-MS of sometimes up to 30-50 VOCs in sediment and biota [95,153,154]. GC-MS is, of course, also the preferred separation technique for the 'old' organochlorines as well as most 'new' organohalogens. However, here the overall picture becomes somewhat more complicated. On the one hand, detection for screening purposes of, specifically, Cl-containing target analytes, still is most conveniently done with an electron-capture detector (GC-ECD). For identification and confirmation, next to conventional GC-MS, the use of ion-trap with its MS^2 option – *i.e.*, increased selectivity – is receiving increased attention. GC-ITMS is a less expensive alternative to high-resolution mass spectrometry (HRMS), which is commonly used to determine PCDD/Fs [155]. For the rest, GC-NCI-MS is a highly rewarding technique for organobromine compounds, toxaphene and organochlorines that contain more that five chlorine atoms. On the other hand, there are also several separation problems – sometimes primarily related to obtaining adequate resolution between analytes and matrix constituents, and sometimes to satisfactorily separating the analytes contained in a priority 'group of substances' from each other. This aspect is briefly discussed in the next paragraphs.

Recent years have witnessed the emergence of so-called comprehensive two-dimensional gas chromatography (GCxGC) – a technique that can be used to considerably improve analyte/matrix as well as analyte/analyte separation. Briefly, a non-polar x (semi-)polar column combination is used, with a conventional 25-30 m long first-dimension, and a short, 0.5-1 m long, second-dimension column. The columns are connected via an

interface called a modulator. The latter device serves to trap, and focus, each subsequent small effluent fraction from the first-dimension column and, then, to launch it into the second column. In order to maintain the integrity of the first-column separation, every peak should be modulated as 3-4 fractions. Consequently, the second-dimension separation has to be a very rapid – and therefore, essentially isothermal – process with a duration of, typically, 3-6 sec. The main advantages of the comprehensive approach are that the entire sample (and not one or a few heart-cuts, as in conventional multidimensional GC [156]) is subjected to a completely different separation, that the two-dimensional separation does not take any more time than the first-dimension run, and that the re-focusing in the modulator helps to increase analyte detectability. A most interesting additional benefit is that structurally related compounds – such as, *e.g.*, PCB or PCDD/F congeners with the same number of Cl substituents – show up as so-called ordered structures in the two-dimensional GCxGC plane. A variety of published papers has shown that this is a powerful tool for the preliminary identification of unknowns [156]. The very rapid second-dimension separation requires the use of detectors with sufficiently high data acquisition rates. Initially, only flame ionisation detectors could meet this requirement. However, today there is also a micro-ECD on the market which is widely used for GCxGC-μECD of halogenated compound classes. Even more importantly, analyte identification can be performed by using a time-of-flight mass spectrometer [157,158] or – with a modest loss of performance, but at a much lower price – one of the very recently introduced rapid-scanning quadrupole mass spectrometers [159,160].

Most studies on ordered structures in the field of organohalogen micro-contaminants deal with PCBs and (priority) PCDD/Fs [161-163]. One interesting observation is that, next to an ordering on the basis of the number of substituents, there is also – within each series of PCB congeners – an ordering due to the substitution pattern, with the non- and mono-ortho congeners, *i.e.* the most toxic ones, ending at the top of the various lines. This provides a better separation from other analytes and facilitates their recognition. Very recently, GCxGC has helped to demonstrate the huge problems still existing in the field of toxaphene and SCCP analysis, already referred to in Section 1.4.3. Partial unravelling of the composition of toxaphene – a technical mixture of, primarily, polychlorinated bornenes, but also bornanes, camphenes and camphanes, has for the first time unequivocally demonstrated the presence of series of penta- to undeca–substituted

congeners, with a total number of individual constituents of over 1,000 [164]. A typical GCxGC chromatogram is shown in Figure 1.10 [164].

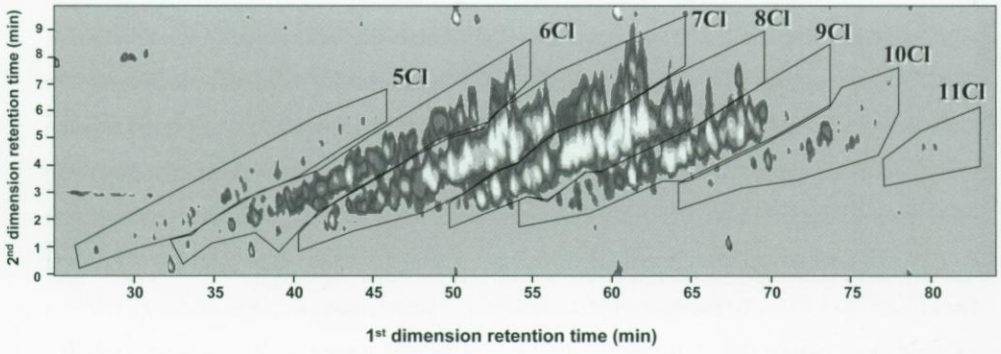


Figure 1.10: GCxGC–TOF-MS total ion chromatogram (*m/z* 45–550) of technical toxaphene. The polygons indicate the congener groups.

An even more complex situation is encountered with the SCCPs. On-going studies [164a] show that what is usually an essentially unresolved large hump in one-dimensional GC, now fans out into a large number of substructures. In this case, there is the additional complication that ordering occurs on the basis of both the number of chlorine substituents and the carbon skeleton length. In other words, whilst indicating that comprehensive separation techniques are demanded to solve analytical problems as outlined here, the example also shows that more effort will be required to arrive at a satisfactory solution, *viz.* the targeted analyses of the most toxic (groups of) congeners and isomers in such mixtures.

For most of the other (groups of) substances included in Table 1.3, there is less need to go into much detail. For example, as regards the majority of the modern pesticides, the phthalate esters and PAHs – which will also preferably be determined by means of GC-based procedures – ample ultra-trace level expertise is available from surface, waste- and estuarine water studies. Limiting ourselves for the sake of convenience to the pesticides, quite a number of papers show that these compounds have been detected down to ca. 1 ng/l concentrations in water form rivers and estuaries. LLE (off-line) and SPE (on-line and off-line) combined with GC-MS or more powerful GS-MS^2 are all suitable techniques

and sample volumes can often be limited to 10-200 ml. A typical advantage of these analytical procedures – and this is also true for most of the LC-MS methods quoted below – is that they have been designed as multiresidue approaches. That is, if in the near future one or more related substances will have to be added to the list, no further method development will be required, but only some (MS) parameter adjustment.

Today, the role of LC – actually, almost exclusively reversed-phase LC – for monitoring and quantifying priority hazardous substances still is fairly modest, but rapidly becoming more important. One rather exceptional example is PAH analysis, where LC provides a more satisfactory overall separation of the target analytes than does GC. An additional benefit is that LC-FLU (fluorescence detection) is a highly sensitive and selective method of analysis for essentially all priority PAHs. With BFRs such as TBBP-A and HBCD, LC-MS is the preferred approach because the analytes of interest easily degrade in GC systems – mainly due to interaction with liners or column walls. In addition, GC cannot separate the three diastereomers of HBCD, while this is easily accomplished on a C_{18} LC-column with a methanol/water gradient [165]. Unfortunately, there is an about 1000-fold loss of detectability compared to GC-MS. At present, the method is therefore suitable only for highly polluted samples such as fatty fish and sediments; for other applications, suitable trace-enrichment procedures will have to be applied. Further, from amongst the modern pesticides, phenylureas such as diuron and isoproturon have to be subjected to LC-MS or LS-MS/MS analysis because of their poor thermal stability and the unsatisfactory nature of most derivatisation procedures. Fortunately, published methods for these pesticides as well as acetanilides, which use SPE combined with LC-ESI-MS/MS, yield fully satisfactory LODs of 0.2-2 ng/l [104].

From among the fluorinated organic micro-contaminants, notably surfactants, PFOS is attracting most attention from environmental scientists. Initially, fluorinated surfactants were analysed by means of GC-MS after derivatisation. Today, direct analysis by means of LC-MS/MS in the ESI mode is preferred [132,166]. The method has been used successfully, down to the low-ng/g level, for biota, sediment and water samples. In seawater, PFOS and several related contaminants have even been determined down to the low pg/l level [167]. For another class of compounds, the alkylphenols and alkylphenol ethoxylates (OP, NP/NPEOs) a rather similar situation is encountered. Earlier GC-MS procedures required derivatisation for all but the most volatile target analytes (and, then,

admittedly, sometimes yield isomer separations not found in LC [117]), and are now being superseded by LC-ESI-MS and -MS/MS which enable the direct determination of all analytes of interest. In this case both normal-phase and reversed-phase LC are used: the former technique allows the separation of the NPEOs according to the number of ethylene oxide units, whilst the latter aims to distinguish the hydrophobic moieties (alkyl homologue separation [117,168]. An interesting discussion of the huge polarity differences of the various types of analytes in the alkylphenol (ethoxylate) classes and the consequences for the preferred mode of MS detection (ESI *vs.* APCI; positive *vs.* negative ion mode) is presented by Reemtsma [169,170]. As with the modern pesticides (*cf.* above), with the quoted multiresidue methods structurally related compounds can be added to the list without a need for further method development. Specifically with the 'emerging pollutants' discussed here, this is a major advantage.

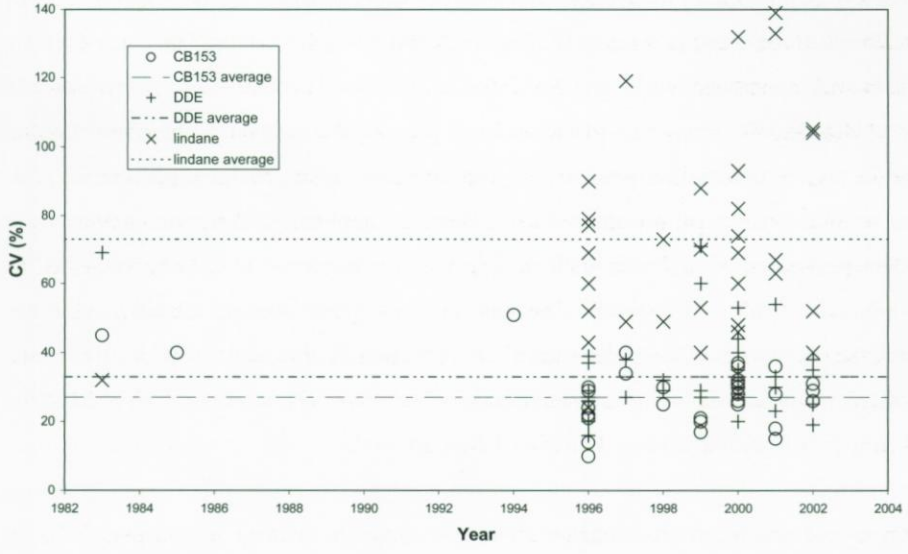


Figure 1.11: Plots of between-laboratory coefficients of variation (CVs) plotted against time for the determination of CB 153, DDE and lindane in various intercomparison exercises (de Boer and Law, 2003).

1.5.3. Quality Assurance

The quality assurance of analytical measurements is, today, receiving increasing attention and will continue to be a most important aspect in the future. To quote an example, a few years ago – *i.e.* during the 1998 OSPAR assessment of trends in the concentrations of

some metals, PAHs and other organic micro-contaminants in the tissue of various fish species and mussels – some 30% of the data had to be rejected because of a lack of, or the insufficiency of, the quality assurance (QA) information [43]. Fortunately, most marine laboratories nowadays are routinely looking at procedural blanks, analysing reference materials and participating in intercomparison exercises. However, this does not invariably mean that the analytical performance of those laboratories has increased as much as one would expect in the past decade. On the other hand, the increased attention to QA does imply that the analytical performance is under control and scientists can, consequently, identify problems and quantify the performance. Recently, de Boer and Law [63] reviewed the analytical performance of laboratories for selected organochlorines and PAHs in the QUASIMEME Laboratory Performance scheme (LPS) and other intercomparison exercises. In this case, the improvement in analytical performance expected because of the evolution of the various analytical techniques could not really be observed. This is illustrated in Figure 1.11 where the between-laboratory coefficient of variation (CV) is plotted against time for some typical OMPs. Even for a well-known compound such as CB 153, the analytical performance has not greatly improved in the last decade and the situation is even worse for lindane. As is well-known, there is an inverse relation between the concentration of the analytes and the CVs. de Boer and Law [63] therefore attributed the lack of improvement to a general decrease in the concentrations of micro-contaminants in the environment and, consequently, in the test samples. However, the question is if concentrations have changed all that much in the recent past. [To our opinion this is not so, as indicated by *e.g.* data on CB levels in cod, flounder, mussel and shrimp for the period 1983-1993 [171] and in cod liver from the North Sea [172]] The authors also stated that, in the past, test samples were often not representative as they had too high concentrations and were therefore less difficult to analyse. For instance, intercomparison exercises for CBs in biota were often run using fish oils that had much higher CB concentrations than fish tissue and are easier to process prior to analysis. This is a more valid argument because the QUASIMEME LPS has switched to real tissue samples in recent years. For lindane this is indeed most probably the major factor contributing to the lack of improvement. The authors also mention that improved statistical methods, which reduce the effect of outliers such as the recently developed Cofino statistics [173], may improve the picture. On the other hand, since marine scientists will continually be confronted with ever-smaller analyte concentrations,

the QA requirements for many compounds included in Table 1.3, will certainly become more demanding.

1.5.4. Environmental variability

In addition to what has been said above about challenges in terms of sample treatment, instrumental analysis and method performance, there is one other item of interest that is, possibly, even more important. Future programmes will have to consider the interconnection between the frequency of sampling, the spatial distribution of sampling stations and the power of a programme to detect, often small, changes of concentrations – *i.e.* trends – in time and space. Specialized OSPAR, ICES and EU working groups are currently discussing these types of questions, but clear advice on *e.g.* the desired degree of spatial and temporal distribution is still not available. Yet, such information is extremely important. For instance, trend detection programmes in OSPAR – and therefore also the statistics – are based on an annual sampling programme for a given region [174,175]. Nicholson *et al.* [176] studied the effect of both environmental and analytical variability on the possibility to detect trends of contaminants in marine matrices. They estimated the performance of a temporal trend programme by calculating the detectable trend, *i.e.* the log-linear trend that would be detected after 10 years with a power of 90% using a test at the 5% significance level. [To quote an example, over a 10-year period a detectable trend of 10% corresponds with an increase of 146% or a decrease of 60%.] This is illustrated in Table 1.6 for what can be called low, medium and high environmental variability. The environmental variabilities in Table 1.6 were based on actual field data on mercury in fish liver for the OSPAR area and include the natural variability as well as variabilities caused by *e.g.* the sampling programmes themselves.

Table 1.6: Trend detectability for varying degrees of analytical and environmental variability (Nicholson *et al.*, 2001).

Environmental variability		Detectable trend (%) for analytical RSDs of:		
Group	**RSD (%)**	**0%**	**12.5%**	**25%**
Low	9.7	3.9	6.5	11.2
Medium	26.2	11	12	15
High	52.4	21	22	24

The authors concluded that if the level of environmental variability is high, trend detection is poor regardless of the level of analytical variability. It is instructive to see that whilst improvement of the analytical performance has a significant effect on trend detectability at low environmental variability, there is essentially no effect in the medium-to-high range. The medium environmental variability calculated here equals the median of that of the mercury data set in the OSPAR database and it is safe to assume that very similar values will be obtained for most areas and contaminants. Indeed, similar variabilities were already calculated by de Boer and Brinkman [177] and could also be calculated in our work on Belgian monitoring data. As a result, one may well conclude that – even with excellent analytical performance characteristics – it will be difficult to detect trends of less than 10-15% over a 10-year period.

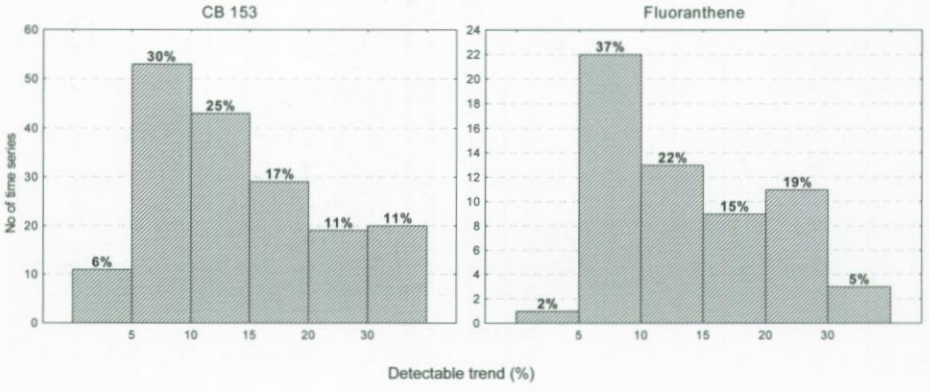


Figure 1.12: Detectable annual trend (indicated in bold) for the time series of CB 153 and fluoranthene in organisms (various fish species and mussels, respectively) from the OSPAR area, calculated for a 10-year dataset [178].

It is interesting to compare the above calculations with a very recent assessment by OSPAR of the data from its CEMP programme (*cf.* Section 1.2) for temporal trends and spatial distribution in organisms and sediments from the OSPAR area [178]. Two illustrative results are shown in Figure 1.9, *viz.* for CB 153 in biota and for fluoranthene in mussels; they are based on a 10-year monitoring study. In both instances, the outcome is seen to agree satisfactorily with the calculations, with the percentages of ca. 60% (less than 15%/year) and ca. 40% (less than 10%/year) for both analytes actually being somewhat better than expected. Since we know that the performance of the analytical procedures has not improved spectacularly in the recent past [63], one tentative

conclusion might be that the environmental variability was 'low' in a large part of the sampling area.

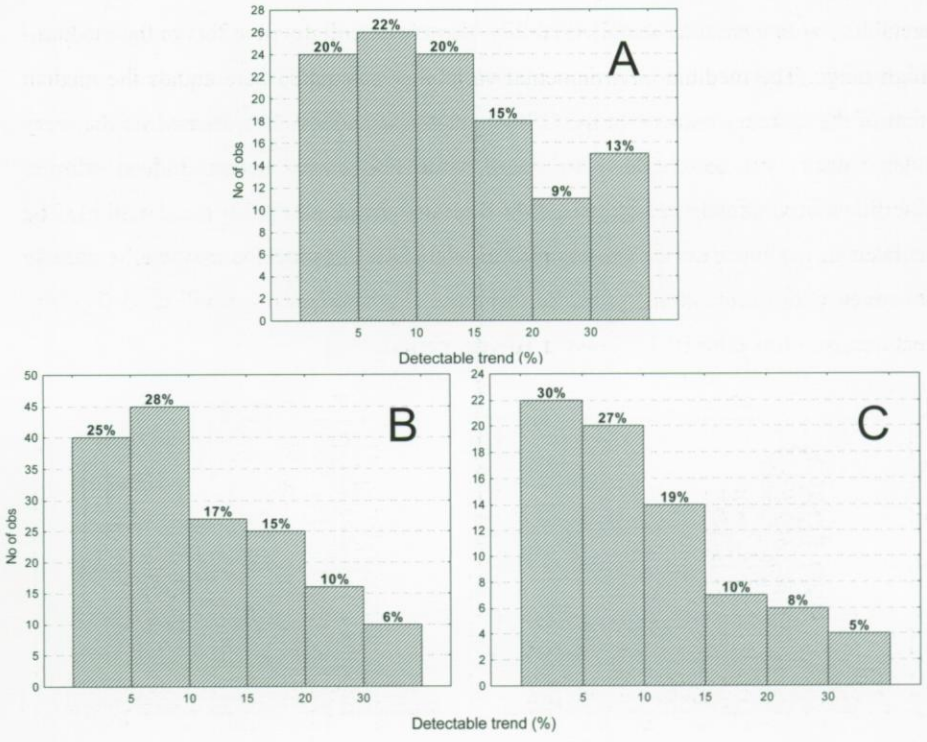


Figure 1.13: Detectable annual trend for the time series of fluoranthene in sediment; (A) not normalised; (B) normalised for C_{org}; (C) normalised for C_{org} and FS all calculated for a 10-year dataset.

For the rest, there clearly is a need to study the environmental variability in more detail. From the literature [171,172,179] it is well known that cofactors such as age, sex, fat content, grain-size distribution, organic matter content, seasonal variations and/or local situations can all contribute to the total variability. In this context, it is slightly surprising that there is, in Figure 1.12, so little difference between the data for CB 153, where data from different organisms were combined as 'biota' and for the much more targeted study on fluoranthene, which was essentially for mussels only. On the other hand, the beneficial influence of normalisation was clearly observed in the same OSPAR-CEMP assessment for, *e.g.*, fluoranthene in sediments (Figure 1.13). Figure 13A shows that, as with the organisms, about 40% of the time series allows trends of less than 10% to be detected (again, a somewhat better results than expected on the basis of the data of Table 1.6).

However, if the time series were normalised for the organic carbon content, C_{org}, and/or the fine fraction (FS) – *i.e.* the fraction <63 µm isolated by sieving – the figures increased considerably, *viz.* to ca. 55% (Figure 1.13 B and C). With the combined C_{org} + FS normalisation, an annual trend of 15% or less can even be detected in three quarters of all time series. To the best of our knowledge this is the first time that environmental scientists have been confronted with sufficiently long and QA-assured time series collected over a wide area and in an international setting which offer the possibility to study the environmental variability and the effect of normalisation in some detail [178]. Consequently, publication of the detailed report of the OSPAR assessment is eagerly awaited.

1.5.5. In summary

- Today there is a large – and to the outsider sometimes bewildering – number of international organizations, national, international, regional and sometimes global monitoring programmes, and increasing activity aimed at cooperation and quality assessment, which all deal with the study (primarily the monitoring) of OMPs in the marine environment. There is a tendency to move towards mutually supporting programmes, but implementation of this concept is still in its infancy.
- Generally speaking, the (groups of) compounds considered priority hazardous substances in most programmes are the 'old' organochlorines, the PAHs and several metals and their compounds. [For the organic pollutants, this emphasis can no doubt be explained on the basis of their adverse PTB characteristics.] In other words, long-term information is limited to these groups. Fortunately, in recent years new and extended lists of OMPs have been published, with newly emerging organohalogens, a limited number of modern pesticides, VOCs, alkylphenols and alkylphenol ethoxylates, SCCPs and PFOS getting a more prominent place. An attempt at 'harmonising' several of such lists to arrive at a tentative OSPAR/North Sea area priority list is included in this review (*cf.* Table 1.3).
- As regards the trace-level determination of the various groups of target compounds in water, sediment and/or biota, a rapid search of the literature reveals that the number of pressing problems is limited. Still, several aspects should be emphasized. While the pre-treatment of aqueous samples is essentially standardized (SPE or LLE approaches), there is much innovation in the field of solid-sample analysis (*cf.* Table 1.5) and further work in this area is recommended. Instrumental analysis is essentially limited to GC-MS (with GC-ECD as a robust alternative for screening of organohalogens) and LC-MS techniques. Specifically the rapid progress in the field of MS-based detection – as manifest from the increasing use of ion-trap, triple-quadrupole and time-of-flight MS – has helped to solve many analytical problems. What will require special attention, next to the ultra-trace-level determination of the priority ortho- and mono-ortho CBs and CDD/Fs, is the analysis of toxaphene (a mixture containing at least 1,000 individual congeners and isomers) and the SCCPs. In both substances, no known GC technique provides the required resolution and comprehensive GCxGC-TOF-MS will have to be used to arrive at something close to a satisfactory solution.

- One positive aspect of the recent wish to start mutually supporting programmes and to pay more attention to data quality, is that intercomparison exercises, quality assurance and proper statistical evaluation of the experimental findings are highlighted in all current activities. As a spin-off, the effects of environmental variability on data interpretation, *e.g.*, in trend analysis and for normalization purposes, are being studied more carefully.
- Finally, government bodies, policy makers and experts involved in setting up programmes as discussed in this review should be aware that monitoring of the marine environment is an expensive business. This is true because of the large cost of sampling at sea but, much more so, because calculations as given above, indicate that in order to detect an annual trend of 10% (a rather high figure in real life), a ten-year sampling programme is required. To add to the worries of those in the office, the OSPAR-CEMP programme was conceived in the early nineties as the result of a discussion that took several years – and it was based on an earlier programme that ran for more than a decade. The OSPAR-CEMP programme, which was modified several times over the years, has been running since then and has now yielded the extensive data set briefly discussed in Section 1.5. That an inception phase of some fifteen years is not unusual is demonstrated by HELCOM. In this case, the convention was signed in 1974, but came into force only in 1980. Monitoring was then started immediately, but it required another seven years before, in 1987, the first assessment of the 5-year period (1980-1985) could be made – thirteen years after the signing of the convention.

1.6 Glossary

ACME	Advisory Committee on the Marine Environment
AEPS	Arctic Environmental Protection Strategy
AMAP	Arctic Monitoring and Assessment Programme
BSC	Black Sea Commission
CEMP	Coordinated Environmental Monitoring Programme
COMMPS	Combined Monitoring-based and Modelling-based Priority Setting
DYNAMEC	Dynamic selection and prioritisation mechanism
EAA	European Economic Area
ED	Esjberg Declaration
EMMA	European Marine Monitoring and Assessment
EMS	European Marine Strategy
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organisation
GEF	Global Environment Facility
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
GIWA	Global International Waters Assessment
GPA	Global Programme of Action
HELCOM	Baltic Marine Environment Protection Commission or Helsinki Commission
IAEA	International Atomic Energy Agency
ICES	International Council for the Exploration of the Seas
IMO	International Maritime Organisation
IMW	International Mussel Watch
IOC	Intergovernmental Oceanographic Commission
JAMP	Joint Assessment and Monitoring Programme
JMP	Joint Monitoring Programme
MEDPOL	Programme for the Assessment and Control of Pollution in the Mediterranean Region
MON	OSPAR Working Group on Monitoring
NGO	Non-Governmental Organisation
NOAA	National Oceanic and Atmospheric Administration
NS&T or NST	National Status and Trends programme
OSPAR	Oslo and Paris Commission
OSPARCOM	OSPAR Commission
PHS	Priority Hazardous Substances
POPs	Persistent Organic Pollutants
PTB	Persistent Toxic and Bioaccumulating
QSR	Quality Status Report
QUASIMEME	Quality Assurance of Chemical and Biological Effects Measurements in Marine Environmental Monitoring
RSP	Regional Seas Programme
SGO	Strategic Goals and Objectives
UNEP	United Nations Environment Programme
UNESCO-IOC	United Nations Educational Scientific and Cultural Organisation - Intergovernmental Oceanographic Commission
WFD	Water Framework Directive
WGSAEM	ICES Working Group on Statistical Aspects of Environmental Monitoring
WHO	World Health Organisation
WMO	World Meteorological Organisation

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1.8 Scope of the thesis

Monitoring plays a vital role in the assessment of human activities which affect the marine environment, particularly with regard to so-called hazardous substances – in this study, organic micropollutants (OMPs). On the one hand, any policy regarding the marine environment should initially be based on an evaluation of the state of that environment. On the other hand, the implementation of a policy must be followed by observing and assessing the evolving situation in the field, as this can have implications for further policy development or adjustment. It is precisely in this context that the work described in this thesis is situated. It deals with three aspects of the activities of a marine chemistry laboratory:

- developing and improving analytical procedures that can detect trace levels of micro-contaminants in the marine environment
- performing baseline studies to assess the potential impact of emerging contaminants
- observing and evaluating the evolving situation of target contaminants in the field.

The general approach and the types of activity used in monitoring studies for the risk assessment of OMPs is illustrated in Figure 1.14 which also indicates the contributions made to this field by the present author.

Chapter 1 discusses the various incentives for and the present framework of marine environmental monitoring. When the widespread pollution by OMPs became globally recognised, monitoring programmes were initiated at both national and international levels. Several important programmes are briefly introduced. These include HELCOM (Baltic area), AMAP (Arctic region) and the global programmes UNEP-POP (the Stockholm Convention), RSP (the Regional Seas Programme) and GIWA (the Global International Waters Assessment). Special attention is devoted to programmes dealing with the North Sea, *viz.* the OSPAR Joint Assessment and Monitoring Programme (OSPAR JAMP) and the Water Framework Directive (WFD). The recently adopted WFD is expected to play an important role in coastal marine environmental monitoring in the near future. The review also discusses the prioritisation mechanisms used to select hazardous and potentially hazardous substances. On the basis of the available evidence,

OMPs which are considered to remain and/or become important for the North Sea area, are highlighted. Several deficiencies of current monitoring programmes are indicated, and improvements are suggested. Due attention is paid to the state-of-the-art of sample preparation and instrumental analysis (almost exclusively gas or liquid chromatography with mass spectrometric detectors), and promising approaches to solve remaining problems are indicated.

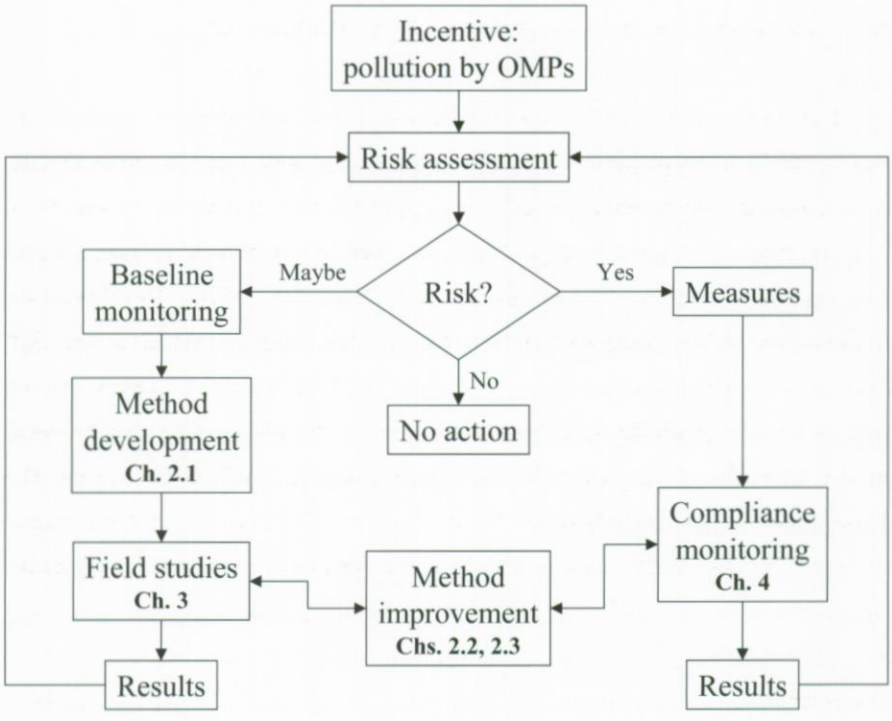

Figure 1.14: General approach and the types of activity used in monitoring studies for the risk assessment of OMPs, including the contributions made by the present author to this field.

Analysis

For many OMPs identified by the prioritisation mechanisms mentioned above, little or no information is available concerning their presence in the marine environment. The volatile organic compounds (VOCs) are a case in point. After the need to obtain experimental evidence on the presence of VOCs in the marine environment and the potential risks for marine wildlife had been voiced, a GC-MS-based analytical procedure

was developed that allows the simultaneous determination of chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene, and the BTEX compounds in marine biota. **Chapter 2.1** discusses how a standard purge-and-trap set-up had to be adapted and re-designed. Particular attention was paid to contamination problems and the general robustness of the P&T-GC-MS method. The newly developed method was used to determine the 13 priority VOCs mentioned above in two fish species from the North Sea. Further technical improvements and the use of a more suitable GC column subsequently allowed the procedure to be applied to some sixty VOCs (**Chapter 2.2**).

Analytical chemistry is a rapidly evolving field and new instrumentation constantly becomes available. It is a major task for a marine chemistry laboratory to keep up to date, *i.e.* to be aware of the potential of evolving methodologies and to implement these whenever appropriate. A good example of this is given in **Chapter 2.3**, where a novel benchtop high-resolution time-of-flight mass spectrometer (TOF MS) was evaluated for the determination of key organic microcontaminants. The major advantage of the TOF MS proved to be the high mass resolution of about 0.002 Da (10 ppm). Consequently, the detectability of polar pesticides, polynuclear aromatic hydrocarbons and polychlorinated biphenyls is excellent, and detection limits are in the order of 1–4 pg injected mass. The high mass resolution is especially useful because it improves the analytical performance when analyzing target compounds in complex samples, and helps to prevent false-positive identifications.

Baseline monitoring

Once a problem has been identified and analytical methods have become available, a baseline survey of concentration levels in the environment is required to help authorities to assess the scope of the problem. For the VOCs mentioned above, initial results already showed that their concentrations in both fish species were at least 100-fold higher than in the surrounding water. Moreover, the highest concentrations detected in these fish were at least ten times higher than those of prominent CB congeners such as CB 153. This caused a more extensive baseline study to be conducted: the results of a 4-year monitoring campaign are discussed in **Chapter 3.1**. During this campaign, the concentrations of the same set of priority VOCs were determined in two species of vertebrates and four species of invertebrates from six sampling stations in the southern North Sea. The initial findings

were confirmed: VOCs were found to be present in all species and at levels which were of the same order of magnitude as previously reported. The concentrations of the chlorinated hydrocarbons (with the exception of chloroform), seem to be lower than those of the monocyclic aromatic hydrocarbons. The data for the latter group of compounds could be linked with the use of fossil fuels. Comparison of the observed concentrations with proposed safety levels showed that the current levels of VOCs will probably pose no acute threat to either man or the fish itself. However, the danger of continuous exposure to these low concentrations of VOCs requires further study. Work reported in **Chapter 3.2** revealed that VOCs generally are not present in sediments from the same area, which confirms that organisms are contaminated through the water column. However, high local concentrations of up to 900 pg/g wwt such as observed in the industrialised area of the port of Antwerp, may be a cause of concern.

The above findings suggest that organisms can be used to monitor the presence of VOCs in the marine environment. A follow-up study in eel from various freshwater bodies illustrates the potential of using organisms as a biomonitor (**Chapter 3.3**). The concentrations in eel are, indeed, a reflection of the actual concentrations in their environment. For fish from the same location similar patterns and concentrations were found, with concentrations that agree with what can be expected from those of the water column. The observed levels could again be linked to the major emission sources and new evidence was presented to show that combustion of fossil fuels is a major source of BTEX in the environment.

Compliance monitoring

In the final chapter of the thesis it is shown how the results of a long-term monitoring programme can be used for scientific purposes. In **Chapter 4.1** CB concentration data for cod, flounder, mussel and shrimp, covering a ten-year period (1983-1993), are assessed for temporal trends and their relation to biological parameters. The study indicates that the lipid content should be taken into account in temporal trend studies because this reduces the differences in CB levels between the organisms and between different tissues within the organisms. The data also show that there is a general downward trend for CB levels on the Belgian continental shelf. In **Chapter 4.2** a similar assessment is made for the concentrations and patterns of CBs in sediments of the Belgian part of the southern North Sea and the Scheldt estuary, in this case for the period 1991-2001. Special attention

was paid to the evaluation of the long-term analytical performance. The study shows how the CB patterns in the fine fraction (<63 μm) of the sediment are closely similar throughout the investigated area. The isolation of the fine fraction by sieving can be regarded as a physical normalisation to reduce differences in sediment granulometric composition. As a consequence, there is a better understanding of CB distribution and patterns and trend analysis is improved. In contrast to the findings reported in **Chapter 4.1** no significant downward trends were observed at any of the twenty-eight sampling stations. This suggests that CB levels have not been changing in the area of interest in the past decade – at least not in sediments. Since the time periods are about ten years apart, and biota and sediments are known to respond differently to pollution, no further conclusions can be drawn by comparing the experimental findings of **Chapters 4.1** and **4.2**.

2 Analysis

2.1 Determination of volatile organic compounds in marine biota*

Summary

A method was developed that allows the simultaneous determination of the volatile organochlorines (VOCs) chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene and the volatile aromatics benzene, toluene, ethylbenzene and the xylenes (BTEX) in marine biota. The biological tissue is first homogenised (at 0°C) using an ultra-turrax blender and transferred to a 25-ml EPA vial. After addition of 15 ml of water and internal standard (1,1,1-trifluorotoluene), the homogenate is treated in an ultrasonic bath (20 min at 0°C) to further disrupt the tissue. The glass vessel is then connected to a Tekmar LSC 2000 purge and trap apparatus coupled to a gas chromatograph-mass spectrometer (GC-MS). The volatiles are forced out of the tissue by purging with a stream of helium gas while heating at 70°C and trapped onto a Vocarb 4000 sorbent trap. After purging, the trap is backflushed while being rapidly heated to 250°C and the analytes are desorbed and, next, trapped in a cryofocusing module (-120°C) connected to the analytical column (Restek, RTx-502.2, 60 m, 0.32 mm i.d., 1.8 μm film). The analytes are injected into the column by rapidly heating the module (from -120°C to 200°C in 0.75 min). Identification and quantification were performed with the mass spectrometer operated in the electron impact mode. The method allows detection limits between 0.005 ng/g (1,2-dichoroethane, 1,1-dichloroethane and tetrachloromethane) and 0.2 ng/g wet weight (chloroform) depending on the background levels and the amount of sample. The reproducibility varies between 8.4% for toluene and 36% for chloroform and the recoveries range from 63% for trichloroethene to 115% for dichloroethane. The method was used to determine the concentrations of VOCs in *Limanda limanda* (dab) and *Merlangius merlangus* (whiting) collected at two sampling stations located on the Belgian continental shelf. Liver and muscle tissue were individually analysed in order to determine the interspecies and interspecimen variability. The results show a considerable variability within tissues of the same species (CV, 50-200%). In most cases, the concentrations of the VOCs appeared to be normally distributed. Although the levels are generally low (low ng/g range), up to 572 ng/g of tetrachloromethane was detected in the liver of whiting.

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2.1.1 Introduction

Oceans and seas are without question the final destination of the greater part of the atmospheric pollutants. Several studies have been dedicated to research concerning the transport, distribution, prediction or measurement of fluxes, and adverse environmental effects of important pollutants such as polychlorinated biphenyls, aromatic hydrocarbons and heavy metals. However, much less is known about the fate of the more volatile species such as benzene, toluene, ethylbenzene, the xylenes and the volatile organochlorines tri- and tetrachloroethylene, di- and trichloroethane and tri- and tetrachloromethane. The latter, which constitute the target compounds of this study, are nevertheless important atmospheric pollutants. They are also part of the group of compounds that have a high research priority according to a number of international organisations [1,2].

Several methods for the determination of volatile organic compounds (VOCs) in biota have been reported in literature. Pearson and McConnel [3] used a Dean and Stark distillation apparatus and *n*-pentane for the extraction of volatile chlorinated compounds from biological tissues. Analysis was then carried out using a gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector (ECD). Gotoh *et al.* [4] crushed the frozen biological material with a mortar and pestle and extracted the volatiles with a mixture of water-*n*-hexane (50/50). The extract was further cleaned up on a micro-Florisil column and analysed by means of GC-ECD. In both cases, the authors reported detection limits in the lower ng/g range which were solely due to the high sensitivity and selectivity of the ECD for halogenated compounds. It would be impossible to reach similar detection limits using the same techniques for non-halogenated compounds such as benzene and toluene.

Ogata *et al.* [5] heated the sample in a gas-tight container at controlled temperatures and sampled the headspace with a heated gas-tight syringe (static headspace technique). The temperature causes the volatilisation of the contaminants from the matrix to the headspace above the matrix. The gas sample is then injected into a GC. As with the previous technique, detection limits often were not satisfactory. The difficulty in applying this technique is also that it relies on the equilibrium partitioning of the analytes between the matrix of interest and the headspace and, thus, relies on the gas/sample partition coefficients. The method should therefore be calibrated for each matrix, which of course is impractical for environmental analysis with its wide variety of samples.

Murray and Riley [6] were among the first to report the determination of VOCs in biota with a dynamic headspace or purge and trap (P&T) technique. Both sediment and biota samples were heated to 200°C in an oven under a stream of purified nitrogen. VOCs were swept away by the nitrogen and trapped on a column packed with 3% silicone oil (SE 52) on Chromsorb W and cooled to -78°C. Next, the trap was allowed to warm to room temperature and the trapped VOCs were injected into the GC with a stream of argon. Hiatt [7] developed a method based on vacuum distillation. The analytes were vaporised from the sample in an ultrasonic bath at 50°C under vacuum and trapped in a super-cooled trap (-196°C) which was essentially a 25-ml purge tube. The concentrator trap was then transferred to a P&T apparatus and desorbed. A major drawback of this system is that it requires the transfer of the purging tube to the P&T apparatus (off-line system). Hiatt [8] further developed the above technique and designed an on-line distillation apparatus. The sample was treated as described above but the cryogenic trap was directly connected to a gas chromatograph-mass spectrometer (GC-MS) system through a double 6-port valve. After purging the cryogenic trap is allowed to warm to room temperature and the analytes are transferred with the carrier gas to a sample loop held at -196°C. The latter is then rapidly heated to 150 °C and the analytes are transported to the GC column by the carrier gas. This technique was further modified [9] by inserting a condenser coil after the sample chamber. This set-up eliminated the need to use a series of temperature baths and facilitated temperature control. Yasuhara and Morita [10] also used steam distillation, in a way similar to Hiatt (1983), for the determination of VOCs in mussel. Easley *et al.* [11] reported a method based on procedures used for water analysis. They developed a purge vessel consisting of a 25-ml glass sample vial and a glass impinger connected to each other by a Wheaton connector. The volatiles were forced out of the tissue by heating the sample to 70°C and purging with a constant flow of helium. The analytes were trapped on a sorbent consisting of equal volumes of Tenax, silica and activated charcoal. After purging, the trap was backflushed with helium and simultaneously heated, and the analytes were transferred to a GC-MS system. Ferrario *et al.* [12] used a system with nitrogen as the purge gas and a Tenax/silica sorbent trap (80/20). Reinert *et al.* [13] described the use of an in-house P&T apparatus. The samples were heated to 50°C by means of a water mantle, purged with a stream of He gas and trapped on an activated carbon trap. The volatiles adsorbed on the carbon trap were then desorbed into a vial containing some carbon disulphide and injected into a GC equipped with a FID (flame ionisation detector). The same authors compared this method to a

procedure involving the use of a grinder/purging apparatus that allowed the grinding of biological tissue and served as the purge vessel at the same time. The tissue was first ground to fine particles in the presence of sodium sulphate. The grinding rod was then lifted to the upper portion of the apparatus and sealed in place with a PTFE O ring. The volatiles were subsequently purged out of the tissue by purging with He at a flow of 100 ml/min for 1 h and trapped onto an activated carbon trap. The latter was then treated in the same way as above.

For this study, we aimed at developing a method that allows the simultaneous determination of halogenated and non-halogenated VOCs in marine biota with a detection limit (LOD) of 100 pg/g or better and with recoveries and a repeatability as good as or better than those reported in literature. Such LODs were, thus far, only reported by authors using an ECD [4] and therefore only for halogenated compounds. Using GC-MS, Easley *et al.* [11] and Hiatt *et al.* [9] reported LODs between 1 and 10 ng/g for both halogenated and non-halogenated compounds and, based on the results and the methodology, it can be expected that Ferrario *et al.* [12] obtained similar LODs. Although all methods could serve as a basis for development, the approach of Easley *et al.* [11] was preferred over the more complicated set-up of and Hiatt *et al.* [9] and the more time-consuming method of Ferrario *et al.* [12]. As Easley *et al.* [11] used a sparging vessel similar to the fritless sparger of our Tekmar LSC-2000 P&T apparatus, the method could be readily adapted to fit the available equipment. For our work, the same type of sparger was purchased and either coupled to the Tekmar (on-line analysis) or used as a stand-alone purging vessel (off-line analysis) or, in other words, the P&T apparatus was used both as an on-line P&T system and as a desorption unit for off-line P&T. After optimisation, the method was applied to the determination of VOCs in two fish species from the Belgian continental shelf.

2.2.2 Experimental

Materials

All materials used for the various experiments and analyses were of research-grade quality. The chlorinated hydrocarbons (CHCs) chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene and the monocyclic aromatic hydrocarbons (MAHs) benzene, toluene,

ethylbenzene and the xylenes were all from Merck (Darmstadt, Germany). They were used without further purification. Methanol (Baker, Instra-analysed, Phillipsburg, USA) was used as solvent for the preparation of standard solutions. 1,1,1-Trifluorotoluene (Aldrich, Milwaukee, USA) was used as internal standard (IS). Vocarb 4000 traps (8.5 cm Carbopack C, 10 cm Carbopack B, 6 cm Carboxen 1000 and 1 cm Carboxen 1001) were obtained from Supelco (Bellefonte, USA) and used as adsorption traps (1/8" OD). Water used for the preparation of blanks and standards was obtained from Baker. Antifoam (Vel, Leuven, Belgium), tungstophosphoric acid hydrate (Merck), sodium chloride (Merck) and 1-octanol (Merck) were used to test the reduction of sample foaming.

Apparatus

A microprocessor-controlled P&T system, the Tekmar LSC-2000 (Tekmar, Cincinatti, USA), was coupled to a GC-MS (Finnigan Magnum Ion Trap MS, Finnigan, San José, USA) via a heated transfer line terminating in a cryogenic focuser at the GC end. The P&T system was provided with a 25-ml fritted sparger and a moisture control module (MCM) as wet trap. The internal lines of the P&T are constructed from glass-lined stainless steel, and the transfer line and internal lines are connected via a heated 6-port switch valve. The samples were purged using an impinger (Alltech, Deerfield, USA) connected to the purge gas outlet and the 25-ml frit sparger of the Tekmar. Prior to analysis, samples were stored in 24-ml sample vials (Alltech). For analysis, they were coupled to the impinger via a Wheaton connector (Wheaton, Millville, USA). Samples were homogenised with a Janke & Kunkel (Staufen, Germany) sharing blender and the tissue was further disrupted in a Bransonic (Branson, Danbury, USA) ultrasonic bath.

Sampling and storage

Fish were caught by the Belgian oceanographic research vessel 'Belgica' at two different locations using beam-trawling and processed as swiftly as possible to avoid contamination and losses. Sampling was done in accordance with the guidelines of OSPARCOM (Oslo and Paris Commissions) [14]. Immediately after sampling, the fish was stored, undissected, at -28 °C in closed containers.

Analytical procedure

Preparation of blanks Water specially prepared for the analysis of VOCs (Baker) was used to prepare blanks and standard solutions (see below). The water was pre-treated by

heating to 90°C with simultaneous purging with helium (N 7.0, l'Air Liquide, Liège, Belgium) or nitrogen (N 6.0, l'Air Liquide) in a glass sparger. As a routine, water used for preparations was continuously purged during storage with the gases mentioned above. For the preparation of blank samples, 15 ml of the treated water were drawn up in a 100-ml syringe and 4 µl of the internal standard were added by inserting a 10-µl HPLC syringe in the opening of the 100-ml syringe. The water sample was then run through the entire analytical procedure, i.e. including homogenisation, treatment in the ultrasonic bath, on-line P&T concentration and GC-MS analysis.

Preparation of standard solutions Methanol was chosen as solvent for the preparation of standard solutions. An initial standard solution (stock solution) was made by diluting 1 ml of the various target compounds in 100 ml of methanol as follows: a small quantity (approx. 20 ml) of solvent was introduced into a volumetric flask and the weight was recorded. 1.00 ml of each of the target compounds was added to the methanol and after each addition the weight was recorded. Finally, the volume was brought to 100 ml and the weight was again recorded to allow correction for possible losses. The procedure enables calculation of the concentration on both a volume and a weight basis. Reporting and using standard solutions on a weight basis is recommended for analytical purposes [15]. However, if volumes are accurately known at the start, concentrations and dilutions can be rapidly calculated. From the stock solution, dilution series were made by dissolving known quantities in methanol, again on a weight basis. Standard solutions were kept in stoppered erlemeyers under methanol. Because of the high volatility of the analytes, frequent renewal of standard solutions is recommended. The diluted solutions were continuously (every 2-3 days) monitored to detect concentration changes. As a rule, no changes of concentration should be allowed that exceed the analytical variability.

For calibration of the procedure, 4 µl of a methanolic solution containing between 0.4 and 0.8 ng/µl of the various target compounds were injected with a 10-µl syringe in an 100-ml syringe containing 15 ml of blank water (see above). Afterwards, another 4 µl of a methanolic solution containing the internal standard (about 0.4 ng/µl) were also introduced into the 100-ml syringe with another 10-µl syringe. The water was then injected into a 24-ml sample vial and the sample vial connected to the on-line P&T set-up, pre-concentrated and analysed by GC-MS.

Table 2.1.1: Retention windows and selected masses of the target compounds.

Compound	Retention window (min)	Selected mass
1,1-Dichloroethane	4:30-4:50	63,64
Chloroform	6:10-6:30	83,85
Trichloroethane	6:40-6:60	61,97,99
Tetrachloromethane	7:00-7:20	117,119
1,2-Dichloroethane	7:10-7:30	62
Benzene	7:10-7:30	78
Trichloroethene	8:00-8:20	60,130
Trifluorotoluene	8:15-8:35	94,129,166
Toluene	9:45-9:65	91
Tetrachloroethene	10:40-10:60	91,105
Ethylbenzene	12:00-12:20	91,106
m&p-Xylene	12:05-12:25	91,106
o-Xylene	12:45-12:65	91,106

Sample pre-treatment and analysis

The frozen fish samples were thawed in their recipients and the edible tissue and liver were isolated from the fish. The biological tissue was first homogenised at 0° C using an ultra-turrax blender (Janke and Kunkel) and transferred to a 24-ml sample vial. After the addition of 10 ml organic-free water and internal standard (1,1,1-trifluorotoluene) the vial was closed with a PFTE-lined screw cap and the homogenate was treated in a ultrasonic bath (20 min at 0°C) to further disrupt the tissue. The glass vessel was then coupled to an impinger connected to the P&T system. The volatiles were forced out of the tissue by purging the sample for 30 min with a stream of helium at 10 ml/min at 70°C (water bath). The analytes were trapped onto a Vocarb 4000 sorbent trap mounted in the P&T apparatus at a temperature of 45 °C. After purging, the trap was backflushed while being rapidly heated to 250 °C and the analytes were desorbed into a cryofocusing module cooled to -120°C and connected to the analytical column. The analytes were injected into the column by rapidly heating the cryofocusing module from -120°C to 200 °C in 0.75 min. Separation was achieved using a 60 m x 0.32 mm i.d. (1.8 µm film) Restek, RTx-502.2 column. Temperature programming of the GC and data acquisition were started simultaneously. The temperature of the GC oven was held at 40 °C for 2 min and then increased from 40 °C to 200 °C at 10 °C/min. This temperature was then held for 5 min. Helium was used as the carrier gas with an inlet pressure of 16 psi. The target compounds were identified on the basis of their retention times and mass spectra and quantified using the total mass of selected ions (2.1.1). The ion trap detector was operated in the electron

ionisation (EI) mode with the multiplier voltage set at 2550 V, the axial modulation (A/M) amplitude at 4.0 V and the emission current at 13 μA. The manifold temperature was set at 220 ° C. The mass range was between 50 - 250 amu and the scan rate 1000 ms. The filament delay was 180 s, and a mass defect of 50 mmass / 100 amu and a background mass of 45 amu were selected.

Analytical quality assurance

A blank sample was run with each series of samples. The peak heights of the analytes in the blank were compared with those in the standard solution used for calibration. Peak heights in the blank should be ten times lower than those in the standard solution (warning limit) and never be less than five times lower (control limit).

A second measure (QA) was to monitor the response factors of the different VOCs during the analysis of the standard solutions used for calibration. Deviations of over 30% from the median response factor were considered as out of control. When the results of a test were out of control, a standard solution was selected and treated as a sample and, thus, analysed as an internal reference material (IRM). The test provides a way to determine whether the problem is MS or P&T related.

Statistical analysis

A Kolmogorov-Smirnov test was executed to determine whether a distribution is normal. The P value of the test was obtained with the Dallal and Wilkinson's approximation to Lilliefors' method [16].

2.1.3 Results and discussion

System blank and removal of excess water.

After the first series of 5-ml blank water samples, consisting of 5-ml water pre-treated as described above, had been analysed, it became evident that traces of chloroform, trichloroethene, benzene, toluene, ethylbenzene and the xylenes were present in the water or in the system, with concentrations ranging from 1 to 20 pg/ml. Neither using different types of water nor extending the pre-treatment procedure of the water (by boiling, extended purging, extraction with hexane or elution over activated carbon) resulted in significant improvements. Desorbing the trap without a preliminary purging step showed the presence of a small quantity of benzene that was thought to originate from the trap

itself. Reconditioning of the trap by baking it overnight at 180 °C indeed resulted in a decrease of the benzene level. This indicated that the water was not the cause of the problem and that the equipment was a more probable source. However, neither thorough cleaning of the system (gas lines, purging device) nor replacing vital components resulted in significant improvements. Running the analytical procedure with an empty sparging vessel gave similar levels of contamination as above, which suggested that the laboratory air might be causing the problems. This was confirmed by the fact that the use of chloroform in another part of the building resulted in increased concentrations of this compound in the blanks. As the purging vessel has to be opened to introduce the sample, contamination by laboratory air could not be avoided.

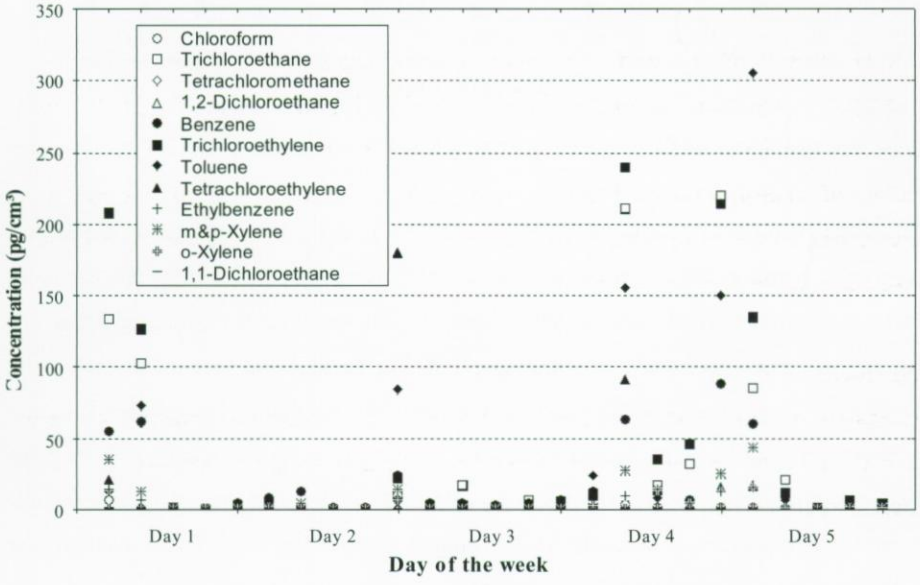


Figure 2.1.1: Analyte concentrations in water blanks recorded over a period of one week.

For a more detailed study of the background levels and their variability, a series of tests was run, that consisted of analysing a series of blank water samples and the concentration in the laboratory air over a period of one week. For the water blanks, internal standard was injected into a luer lock syringe filled with blank water (5 ml) and analysed according to Easley *et al.* [11]. The background concentrations in air were determined by analysing the air in an empty sparging vessel of the Tekmar, as above.

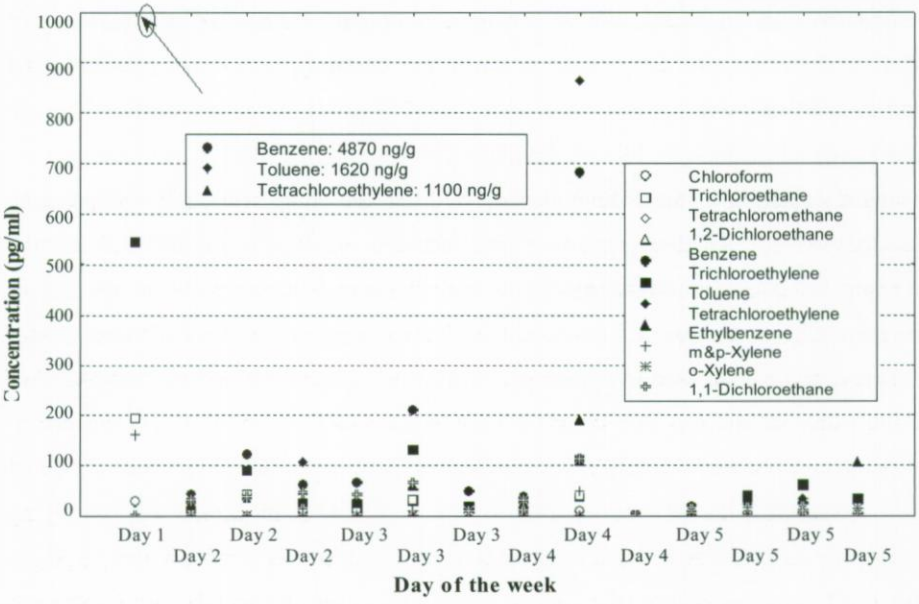


Figure 2.1.2: Variability of the background concentration in laboratory air over a period of 1 week.

The results of these tests are shown in Figures 2.1.1 and 2.1.2. As regards the water, background levels seem to be below 200 pg/ml, with some high values for benzene, toluene and tetrachloroethylene as exceptions. Levels reported for drinking water are generally at or above these concentrations [17]. No background levels (i.e. below 10 pg/ml) were found for 1,2-dichloroethane and tetrachloromethane. The relative standard deviation (RSD) of the experimental results for the various compounds ranged from 20 to 120% when the outlying values (sequence numbers 1 and 9) were excluded. The within-day variability (sequence numbers 11-14) ranged from 20 to 70%. If the background should be due to contamination by laboratory air, the concentrations plotted in Figure 2.1.1 should be divided by 4 or, in other words, the headspace concentrations should be below 50 pg/cm³. The results of Figure 2.1.2 indeed indicate that the levels in laboratory air are around that level with average concentrations ranging from 10 to 70 pg/cm³. Frank *et al.* [18] reported levels of CHCs in air ranging from 20 to 300 pg/cm³ at the Atlantic coast of Portugal, while Bianchi and Varney [19] reported levels up to 16 ng/cm³ over the Southampton Estuary. Levels for MAHs in air at the Bretagne coast (France) ranged from 20 to 600 pg/cm³ [20] and from 1 to 200 ng/cm³ in the Southampton Estuary [19]. It can therefore be assumed that the presence of volatiles in laboratory air is largely responsible for the observed background levels. This indicates that the contact time between sample

and laboratory air and the headspace above the sample should be kept at a minimum. It also means that background concentrations must be constantly monitored by performing a blank analysis each day.

Prolonged operation of the system also showed that the equipment itself was prone to contamination by samples containing high amounts of VOCs. To our opinion the switching valve and the automatically controlled opening valves serve as sources of adsorption and, consequently, contamination that can never be entirely eliminated with the present equipment. During these initial tests, it also became evident that the MCM of the P&T did not succeed in retaining all the water vapour released during purging at elevated temperatures (70 °C). The ion trap became saturated with excess water that could only originate from the P&T device. Eliminating the water vapour formed during purging was therefore a prerequisite for a proper analysis. To remedy the problem, the purge gas was passed through a glass cooler kept at –10 °C. However, this had the disadvantage of frequent clogging of the line due to the formation of ice. As an alternative, the sparger of the Tekmar was filled with water kept at room temperature, in order to condense the water vapour present in the purge gas. Water was then further eliminated in the MCM. Although this procedure turned out to be successful, operation during extended periods of time sometimes resulted in the breakthrough of water. The system was then purged overnight at elevated temperature.

Sample treatment and foaming

Sample treatment involves dissection of the organism to obtain the edible tissue and processing of the tissue. Sample treatment described in the literature involves homogenisation [3,8,10], ultrasonication [11] or a combination of both [12]. Samples are generally treated at low temperatures (from 4 °C to cooling with liquid nitrogen). Homogenisation of the tissue improves passage of the purge gas and thus, volatilisation of the analytes, but increases the risk of losses of those analytes and, contamination. Ultrasonication causes an intense cell disruption and is thought to enhance the release of trapped volatiles in the cells while they still remain in the matrix [11]. For our work, a combination of tissue homogenisation with a sharing blender at 0 °C and treatment in an ultrasonic bath gave the best results. Comparing the above combination with ultrasonication alone showed a similar analyte yield for most VOCs, but much better RSDs for the former procedure (Table 2.1.2). Moreover, trichloroethylene could

apparently only be purged out of the tissue after homogenisation and there was a much higher yield for chloroform.

Table 2.1.2: Effect of homogenisation on the analysis of fish tissue samples.

Compound	Not homogenised (n=5)		Homogenised (n=5)	
	Average (ng/g)	RSD%	Average (ng/g)	RSD%
Chloroform	3.2	43	13.62	21
Trichloorethane	0.19	47	0.05	18
Tetrachloromethane	<0.005	-	<0.005	-
Benzene	1.9	14	2.89	20
Trichloorethylene	<0.02	-	5.53	10
Toluene	2.5	57	1.20	28
Tetrachloorethylene	5.3	45	2.07	19
Ethylbenzene	2.4	50	1.70	9
m&p-Xylene	2.5	25	2.96	15
o-Xylene	1.4	53	1.44	13

n = number of analyses, RSD = relative standard deviation,

Initially, severe sample foaming was observed. This is the result of denaturation of proteins at the elevated temperatures used during purging and can cause deactivation of the trap and or contamination due to the introduction of thermal decomposition products from labile, non-volatile materials. Sample foaming can be reduced by decreasing the purge flow or by inserting a mechanical barrier, such as glass wool in the purge vial. Easley *et al.* [11] observed no foaming at flow rates of 40 ml/min and with the equipment they used. However, using similar conditions, a glass wool barrier could not containin the severe sample foaming in our work which agrees with the findings of Michael *et al.* [21]. Using an antifoaming agent as an alternative we observed the occurrence of a number of unknown interfering peaks in our chromatograms as has also been observed for a similar antifoaming agent (Dow Corning antifoam [21]). The use of up to 10 wt.% tungstophosphoric acid hydrate in water did not effect a sufficient reduction of sample foaming and the use of a heatgun [22] was neither efficient nor practical. Finally, the purge flow was gradually reduced to a rate that gave little or no sample foaming, with a purge flow of 10 ml/min giving good results. In combination with glass wool as a mechanical barrier this eventually turned out be the best way to prevent foam from reaching the trap.

Unfortunately, reducing the purge flow had serious implications with regard to the time needed to force the analytes out of the sample. Most authors report purge flows above 30 ml/min and purge times that vary between 12 min and 2 hours with the actual values depending on the volume of the purging device [11,12,13]. In order to optimise the purge time and flow conditions, a homogenised whiting muscle sample was ultrasonicated for 2 min and purged with a flow of 10 ml/min (cf. above) using increasing purge times. Real samples were preferred over spiked ones, because the former would give a more realistic picture of the amount of analytes forced out of the tissue. Peaks were considered significant when their height exceeded twice that of the analytical blank. The experiments revealed that a significant proportion of all analytes was released within 20 with an optimum around 40 min for the MAHs and around 30 min for the CHCs (Figure 2.1.3). For the simultaneous determination of both groups of compounds a purge time of 30 min was selected.

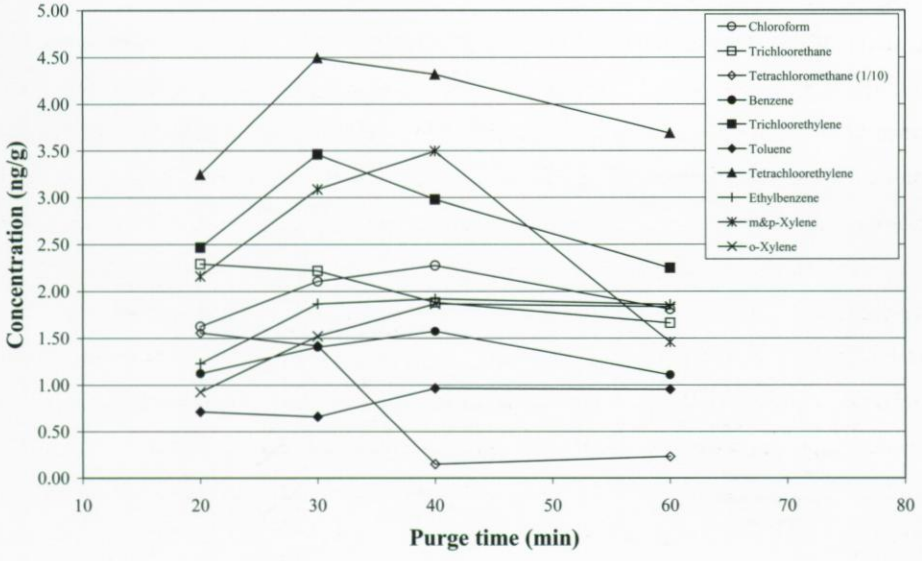


Figure 2.1.3: Dependence of recovery on purge time.

Comparison between off-line and on-line determination

All analyses were initially performed off-line. To study the feasibility of on-line P&T, the vessel used for the off-line determination was coupled to the sparger of the Tekmar (Figure 2.1.4). Since this is the only change in the set-up, the experimental conditions could be kept the same. The main focus was therefore on the background obtained with the on-line system. To this end, a blank water sample was analysed several times using both set-ups. The experimental results are shown in Table 2.1.3; the peak areas of the internal standard were the same in both methods. The blank values are in general significantly lower when using the on-line method, with the exception of benzene, which originates from the trap (cf. above), and trichloroethylene. However, the peak area of the latter was very small and the compound was only detected on two occasions, with one high value explaining the dramatic RSD value for the on-line method. For all other analytes, the precision was the same or much better with the on-line set-up. As a result, it was selected for all further work.

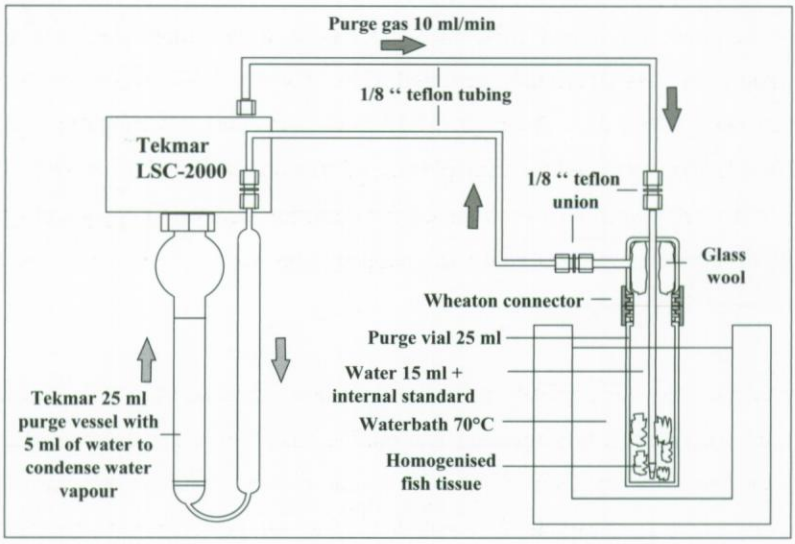


Figure 2.1.4: Schematic of the on-line set-up.

Table 2.1.3: Average peak height, relative standard deviation (RSD) and ratio between the averages of analyses blank water with the off-line and on-line set-up.

Compound	Off- line (n=6)		On-line (n=5)		Ratio
	Average	RSD%	Average	RSD%	
Chloroform	210000	37	1900	36	114
Trichloroethane	2900	14	0	-	>>
Tetrachloromethane	nd	-	nd	-	-
Benzene	265000	41	349242	16	0.7
Trichloroethylene	1600	26	1044	127	1.5
Toluene	183000	37	20603	12	9
Tetrachloroethylene	48700	36	nd	-	>>
Ethylbenzene	196000	30	4993	12	39
m&p-Xylene	284000	26	11037	17	26
o-Xylene	66000	25	1413	20	47

n = number of analyses, ratio = average off-line/average on-line, nd = not detected

Analytical data

The limit of detection (LOD) of the VOCs was calculated using two methods. Considering the variability of the daily blank values, the limit of detection was defined as the amount corresponding to the blank plus three SDs of the blank. Since, in practice, the RSDs of the blank are around 30%, the LOD was set at two times the blank value. A similar approach was previously reported [23]. For the compounds for which no significant blank levels were observed, the LOD was set equal to a signal-to-noise (S/N) ratio of 3:1. LODs calculated for a sample size of 10 g are presented in Table 2.1.4. They range from 5 to 200 pg/g. This work aimed at a detection limit of 100 pg/g wet weight or better. With one exception this goal was obtained, with half of the values being even 5-20-fold lower.

In order to determine the repeatability or the short-term variation of independent analyses of the total procedure, a homogenised fish muscle tissue sample was prepared and five separate analyses were performed with the on-line method. The test was limited to the repeatability of the same sample. Five samples is the average number of samples that can be analysed in one day. A day to day approach is to be preferred because varying background levels will largely influence the long-term reproducibility. Samples were therefore processed batchwise in such a way that each batch could be analysed in one day, together with the required blank and standard runs. The results are given in Table 2.1.4. The RSD values varied between 8% and 25% for all VOCs except chloroform (36%). RSDs reported in the literature for the various methodologies vary between 2 and 30%,

and, specifically for purge and trap techniques, RSDs are between 5 and 20%. The rather close similarity, between the various sets of RSDs strongly suggests that they are the best available for P&T techniques today. Since the results moreover comply with the goals set at the beginning of this work, no further effort was made to improve the repeatability.

Table 2.1.4: LODs, within day precision and recovery for the analysis of VOCs in 10 g marine biota samples.

Compound	LOD (pg/g)	RSD (%) (n=5)	Recovery (%) (n=5)
Chloroform	200	36	95 ±36
1,1,1-Trichloroethane	6	24	66 ±24
Tetrachloromethane	5	24	70 ± 24
Benzene	80	16	80 ± 18
Trichloroethylene	20	16	63 ± 17
Toluene	80	8	115 ± 11
Tetrachloroethylene	60	11	74 ± 11
Ethylbenzene	20	11	72 ± 15
m&p-Xylene	80	12	69 ± 15
o-Xylene	20	21	77 ± 25
1,2-Dichloroethane	5	25	115 ± 25
1,1-Dichloroethane	5	25	115 ± 25

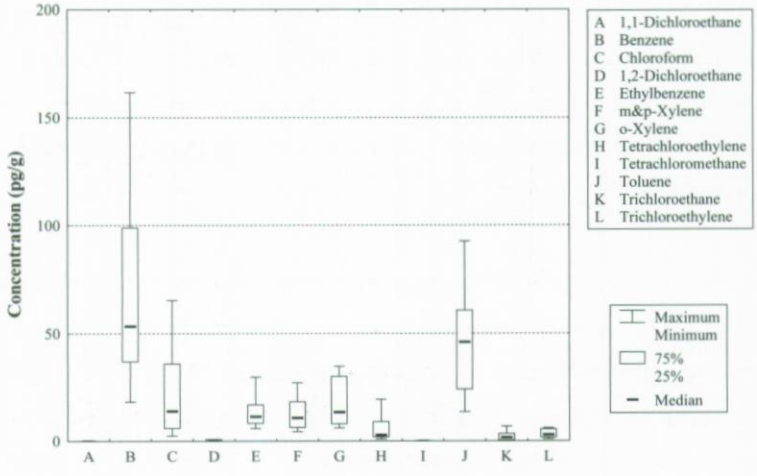


Figure 2.1.5: Background concentrations of analytes of interest over a period of 22 weeks.

To determine the recovery, a homogenised fish muscle tissue sample was prepared and divided into five parts. The homogenised samples were immediately transferred to sample vials and water was added which contained both the internal standard and a known concentration (about 1 ng/g) of the target compounds. The samples were stored for 24 hours at room temperature and in the dark prior to analysis. The experimental results of the analysis are included in Table 2.1.4. The recovery is seen to vary between 63% for trichloroethylene and 115% for toluene and the dichloroethanes. Recoveries reported in the literature range from 46 to 129% for the various techniques and from 60 to 90% for the purge and trap techniques. Or, in other words, the recoveries obtained with the discussed procedure are similar to those reported by other authors. However, here one should consider that, in order to collect data close to the LODs aimed at (cf. above), in this study samples were spiked at concentrations of about 1 ng/g as against 20 to 2000 ng/g in earlier work. This helps to illustrate the good performance of the present procedure.

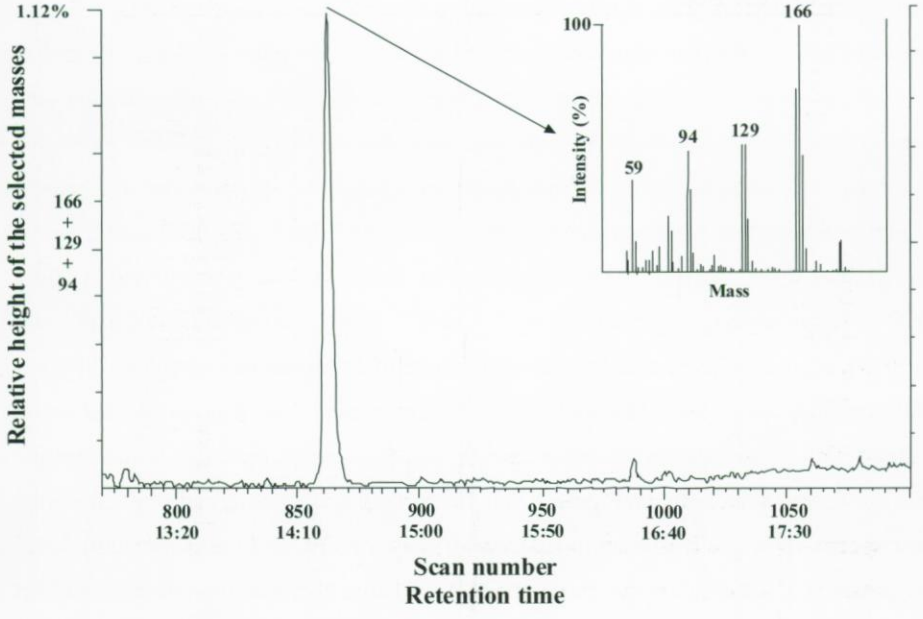


Figure 2.1.6: Selected ion chromatogram and mass spectrum for tetrachloroethylene (80 pg/g) in muscle tissue of whiting.

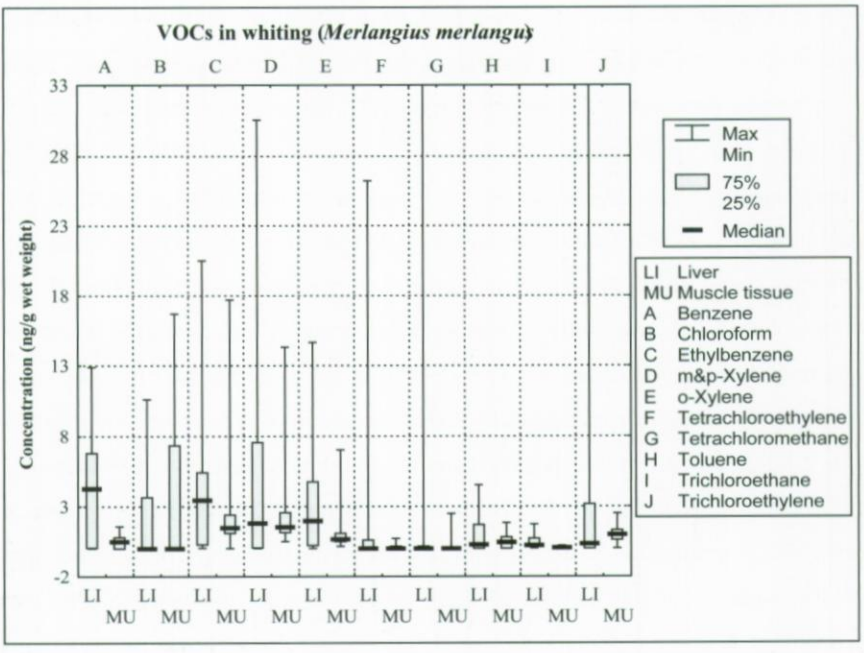
Analytical quality assurance

A blank analysis accompanied each batch of samples. The long-term variability of the blank during a period of 22 weeks is illustrated in Figure 2.1.5. With the exception of some extremes (defined as 3 times the difference between the 75th and 25th percentiles) and outliers (defined as 1.5 times the difference between the 75th ad 25th percentiles) the concentrations of the blanks generally were below 100 pg/g and even below 50 pg/g for the CHCs. For 1,1-dichloroethane, 1,2-dichloroethane and tetrachloromethane, the blank values generally were below the detection limit. In other words, as a concentration of about 1 ng/g is conventionally used for calibration purposes, the background concentrations typically were more than ten times lower. The higher values found for the MAHs can probably be attributed to the Tekmar P&T apparatus which is prone to becoming contaminated with MAHs. The higher values for chloroform compared with the other CHCs were no doubt due to the frequent use of this solvent in other parts of the building.

Positive identification of the target compounds was performed using both their retention times and MS spectra. The high sensitivity of the ion trap MS allows full-scan spectra to be recorded even at low concentrations. The procedure for the identification and quantification of the target compounds was as follows. First the absence / possible presence of a compound was established by observing the absence / presence of a peak in the pertinent retention window using the selected ions of Table 1. If a peak was detected, the full-scan mass spectrum was compared with that in a home-made library (Figure 2.1.6). For our purposes, the minimum fit (scale 0-1000) was set at 700. Small mass fragments are always present in the full-scan spectra of environmental samples. The latter influence the fit value and a minimum fit of 700 therefore seemed appropriate. However, in about 90% of all cases in which an analyte was detected on the basis of its retention time, the fit was 800 or above (Figure 2.1.6). There were two types of exception, distorted mass spectra as a result of water breakthrough (99%) and co-eluting compounds (1%). The presence of water in the ion trap of the MS will cause chemical ionisation. The effect will be observed at the level of the molecular mass ion (M) and will result in the presence of a strong M-1 mass ion peak. As quantification is based on the most prominent mass ions (Table 2.1.1), a shift will make accurate calculation of the concentration impossible. If such an untoward event occurred, the analysis was halted and the system was purged overnight at elevated temperature (250 °C). Re-running the sample afterwards invariably

led to positive identification. In a very few cases, additional masses were detected in the spectrum. The ratios between the most prominent masses of the target compound were then used to determine whether a coeluting compound was present or the peak should be considered as a false positive. Positive identification, i.e. quantification, was made only if the relevant peak ratios matched those of the library spectrum within 20%. The present on-line procedure was used to determine VOCs in marine biota. Two fish species, whiting (roundfish) and dab (flatfish), were collected at two sampling stations on the Belgian continental shelf. About 25 specimens of the same length class were collected for each species and analysed individually. Apart from this being a field test for the procedure, the monitoring operation provided a way to establish the concentrations of VOCs actually present and the range of concentrations within a population. The results are illustrated in Figure 2.1.7. Although, the range of concentrations within a tissue was considerable, with coefficients of variation (CVs) varying between 20 and 200%, the concentrations of the various VOCs appeared to be normally distributed in the tissues of specimens originating from the same population (Table 2.1.5). The latter can be explained on the basis of individual specimens within the same population being affected by the same source. Since they can be expected to accumulate or eliminate the chemical in similar ways, the net result would therefore be a normal distribution within in a population. A distinct difference was noted between the results found for liver and muscle tissue of both fishes. Liver generally contained higher concentrations of the VOCs than muscle tissue. This can be explained by the fact that exogenous compounds are mainly metabolised in the liver. Only for chloroform, higher concentrations were found in muscle tissue. Since both species store their lipid reserves in the muscle tissue and lipid-soluble compounds that are not excreted or metabolised are mainly stored in the fat reserves of the organism, this is not unexpected. Indeed, a significant relation could be demonstrated between the concentration of chloroform and the fat content of muscle tissue (Figure 2.1.8).

The presence of VOCs in biota was reported as early as 1975 [3]. However, only a few authors have reported quantitative data. The concentrations found during this study were similar to those previously published. For whiting, average concentrations of 1-2 ng/g wet weight (wwt) were found for muscle tissue and around 1-6 ng/g wwt for liver tissue.



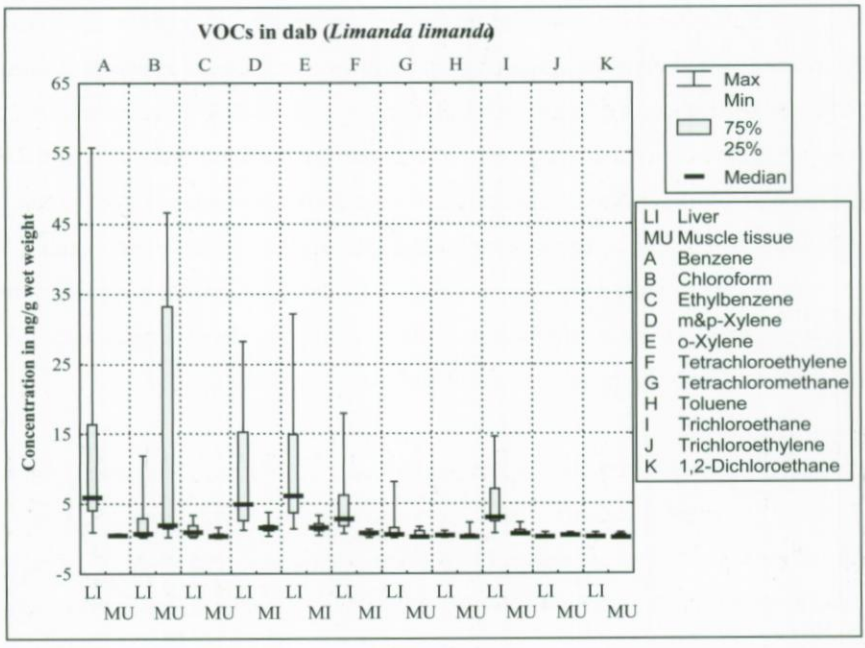


Figure 2.1.7: Concentrations of VOCs in muscle tissue and liver of whiting (top) and dab (bottom). Values below detection limit are included in the plots.

Table 2.1.5: Statistical analysis of VOCs in muscle and liver of whiting and dab and correlation with fat content and length.

Parameter	n	Median (ng/g)	Mean (ng/g)	CV (%)	KS distance	KS P-value
			Whiting muscle tissue			
Benzene	13	4.4	4.0	46	0.151	>0.10 *
Chloroform	10	42	198	83	0.417	0.062 *
m&p-Xylene	20	6.4	13.9	139	0.372	0.0079
Ethylbenzene	21	5.7	10.8	117	0.322	0.0257
o-Xylene	21	3.1	8.6	126	0.386	0.0039
Tetrachloroethylene	12	0.2	0.3	64	0.244	>0.10 *
Tetrachloromethane	4	0.7	3.2	191	0.418	>0.10 *
Toluene	15	2.6	3.4	63	0.188	>0.10 *
Trichloroethane	9	1.0	1.2	79	0.268	>0.10 *
Trichloroethylene	20	4.6	4.6	58	0.127	>0.10 *
			Whiting liver tissue			
Benzene	18	4.9	6.0	68	0.146	>0.10 *
Chloroform	11	3.7	4.7	78	0.155	>0.10 *
m&p-Xylene	23	3.5	4.9	126	0.247	>0.10 *
Ethylbenzene	19	3.4	6.7	134	0.232	>0.10 *
o-Xylene	21	2.2	3.6	114	0.220	>0.10 *
Tetrachloroethylene	9	0.89	4.3	203	0.420	0.0839 *
Tetrachloromethane	10	76	154	128	0.283	>0.10 *
Toluene	12	1.6	2.1	68	0.195	>0.10 *
Trichloroethane	20	0.22	0.5	103	0.256	>0.10 *
Trichloroethylene	16	1.2	29	195	0.411	0.0091
			Dab muscle tissue			
Benzene	16	0.5	0.54	20	0.158	> 0.10 *
Chloroform	17	1.9	14	126	0.329	0.0502 *
m&p-Xylene	16	0.4	0.52	86	0.187	> 0.10 *
Ethylbenzene	18	1.7	1.71	47	0.145	> 0.10 *
o-Xylene	16	1.0	1.75	43	0.181	> 0.10 *
Tetrachloroethylene	16	0.8	0.77	42	0.111	> 0.10 *
Tetrachloromethane	13	0.3	0.62	92	0.315	> 0.10 *
Toluene	12	0.3	0.54	113	0.261	> 0.10 *
Trichloroethane	14	0.7	0.97	53	0.260	> 0.10 *
Trichloroethylene	6	0.5	0.53	39	0.313	> 0.10 *
			Dab liver tissue			
Benzene	19	5.6	12	116	0.257	> 0.10 *
Chloroform	20	0.8	2.5	136	0.272	> 0.10 *
m&p-Xylene	16	1.0	1.3	78	0.157	> 0.10 *
Ethylbenzene	20	4.9	9.4	95	0.250	> 0.10 *
o-Xylene	20	6.2	10	90	0.201	> 0.10 *
Tetrachloroethylene	20	2.8	4.8	96	0.273	> 0.10 *
Tetrachloromethane	20	0.7	1.3	137	0.273	> 0.10 *
Toluene	8	0.5	0.6	55	0.215	> 0.10 *
Trichloroethane	20	3.0	4.9	77	0.224	> 0.10 *
Trichloroethylene	17	0.3	0.4	69	0.269	> 0.10 *

n = number of values used for the calculation (i.e. number of fish and exclusion of values below detection limits), CV = coefficient of variation, * = significant, KS = Kolmogorov-Smirnov.

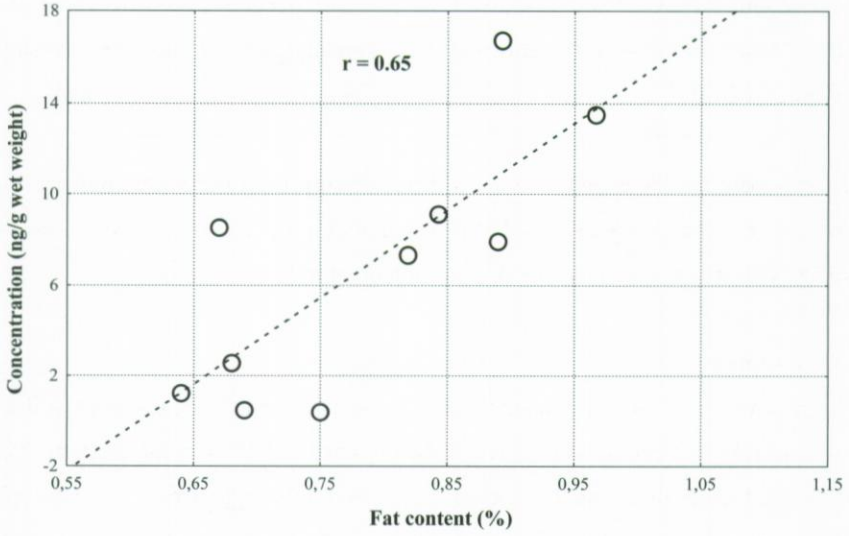


Figure 2.1.8: Relationship between chloroform concentration and fat content in muscle tissue for whiting. See text for details.

Similarly, concentrations of several CHCs averaging around 5 ng/g wwt were reported for a related species like cod (*Gadus morhua*) [3,24]. For dab, the average CHC concentrations were between 0.3 and 2 ng/g wwt for muscle tissue and between 0.3 and 6 ng/g wwt for liver tissue. Similarly, Pearson and McConnel [3] reported concentrations of 1-20 ng/g wwt for muscle tissue and of 12-30 ng/g wwt for liver tissue. Concentrations in related fish species like plaice (*Pleuronectes platessa*) and flounder (*Platychtis flesus*) were of the same order of magnitude. The highest concentrations observed during this study were over 150 ng/g wwt for trichloroethylene and over 550 ng/g wwt of tetrachloromethane in liver of whiting. Similarly high values have been reported in the literature in a few examples, although mostly for invertebrates. Yasuhara and Morita [10] found 4080 ng/g wwt of 1,2-dichloroethane in mussel (*Mytilus edulis*) while Reinert et al. [13] reported 590 ng/g wwt of the same compound in grass shrimp (*Paleomonetes pugio*). Pearson and McConnel [3] found up to 150 ng/g wwt of chloroform in cockle (*Cerastoderma edule*), and 180 ng/g wwt in crab (*Cancer pagarus*). Ferrario et al. [12] reported benzene concentrations up to 1030 ng/g in killifish (*Fundulus sp.*). Finally, the concentrations reported in this study are of the same order of magnitude as those found for chlorinated biphenyls (CBs) in fish caught in the same area [25]. CBs have a high

octanol-water partition coefficient and therefore a strong tendency to bioconcentrate. It is generally expected, however, that volatile organic compounds exhibit little or no tendency to bioconcentrate [17].

The similarity between the concentrations is therefore surprising and would indicate that the organisms are either exposed to higher levels of VOCs or, at least, more frequently to lower levels. This aspect certainly merits closer attention in future studies.

2.1.3 Conclusions

Even though several sets of experimental data have been quoted above, one may well say that there is a lack of attention with regard to the presence of VOCs in marine biota. This is often attributed to their low bioconcentration and bioaccumulation potential [3], especially in comparison with hydrophobic compounds such as CBs. Yet the VOC concentrations in both fish species are at least a 100-fold higher than in the surrounding water. Dewulf and Vanlangehove [26] reported concentrations ranging between 10 and 50 ng/l for MAHs and 1 and 20 ng/l for CHCs in water from the same area. Moreover, the extremes in the fish species were at least ten times higher than those of prominent CB isomers, such as IUPAC Nr.153 [27], which are priority pollutants. Admittedly, most environmental pollutants are present at levels that will not result in acute toxic effects and the current levels of VOCs will probably pose no threat either to man or the fish itself. However, the danger is the continuous exposure of organisms to these compounds and the present concern is indeed focuses on the effect of a long-term exposure to low levels of contaminants [28]. VOCs are obviously present at such concentrations and a thorough knowledge of their presence and distribution is mandatory for an accurate risk assessment. In that respect, a rather rapid and sufficiently sensitive and selective way to determine both MAHs and CHCs in marine biota is provided by the current procedure.

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2.2 Improved determination of VOCs in marine biota by using on-line purge and trap–gas chromatography–mass spectrometry[‡]

Summary

A Tekmar LSC-2000 Purge and Trap (P&T) apparatus was further modified in order to improve the on-line P&T gas chromatographic determination of Volatile Organic Compounds (VOCs) in biological tissue. The standard needle sparger of the Tekmar was replaced by a system consisting of two needles (purge gas in- and outlet) and a moisture trap. This modification allows a rapid throughput of samples and minimises the risk of contamination or losses. Addition of 1-octanol proved successful in eliminating the severe sample foaming that generally occurs when biological material is purged. For separation of the analytes a J&W DB-VRX column (60 m, 0.25 mm i.d., 1.4 μm film) was used, which allowed the elimination of the cryofocusing step prior to injection. The method was tested for 13 priority VOCs and detection limits were obtained ranging from 0.003 ng/g (tetrachloromethane) to 0.16 ng/g (*m*- and *p*-xylene) using single ion monitoring-mass spectrometry. The reproducibility was around 15% for most compounds and the recoveries were better than 80% for all analytes except 1,1-dichloroethane (59%). Although the method was originally validated for 13 VOCs, it was found to be applicable for a broader range of VOCs and was tested on eel from the Scheldt estuary. Apart from the priority VOCs several other VOCs turned up rather unexpectedly in these samples. They were identified on the basis of their mass spectra and quantified using selected ion monitoring.

[‡] From *Analyst*, 123 (1998) 2167-2173.

2.2.1 Introduction

There is relatively little information on the presence and distribution of volatile organic compounds (VOCs) in marine organisms. This is in part due to the assumed low potential of the VOCs to bioconcentrate [1] and possibly also to the analytical difficulties that are encountered in this type of monitoring. Most VOCs are nonetheless important atmospheric pollutants and a number of them are recognised as compounds with a high research priority by several international organisations [2-4]. A limited number of authors [5-8] have reported the presence of VOCs in marine organisms, some of them as early as 1975. Recent work revealed the presence of VOCs in marine organisms at levels at or above those of well-known contaminants such as PCBs [9]. So far, the implications for marine organisms are unknown. As for PCBs the levels are such that there will probably be no acute effects for organisms and man, but the effects of long-term exposure are of some concern.

VOCs are determined in organisms using sample-treatment techniques such as solvent extraction [5,8], static headspace[10], vacuum distillation [11-13] and purge and trap (P&T)[14-16]. The lowest detection limits are generally reached with those methods that use dynamic headspace techniques (vacuum distillation, P&T). They are less matrix dependent than static headspace techniques and are readily used in combination with gas chromatography-mass spectrometry (GC-MS). Using such an analytical technique detection limits (LODs) better than 100 pg/g have been obtained [9].

In an on-going effort to study the concentrations of VOCs in organisms, a previously reported methodology [9] was further improved. Although the latter was successfully used for the determination of VOCs in organisms, a number of shortcomings gradually became apparent. The P&T set-up was prone to leaking, especially after extended periods of operation. Furthermore, samples had to be exposed to ambient air, although briefly, when sample vials were coupled to the system, which always involves a risk of sample contamination or analyte losses. Even at the low purge flows used, excessive sample foaming sometimes occurred and inevitably resulted in contamination of the system and, consequently, system down time. The current work therefore aims at improving the robustness of the method for use in a more routine environment. The method was tested by exploring the possibility of determining a larger number of VOCs in biota. In anticipation of a planned monitoring programme for yellow eel (*Anguilla anguilla*), eel

from the Scheldt estuary were used for this purpose. Eel is regarded as an excellent biomonitor for fresh water systems because of its non-migratory behaviour, high fat content, wide distribution and absence of spawning [17].

2.2.2 Experimental

Reagents and chemicals

All materials used in this study were of research-grade quality. The chlorinated hydrocarbons (CHCs), chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene and the monocyclic aromatic hydrocarbons (MAHs), benzene, toluene, ethylbenzene and the xylenes were all from Merck (Darmstadt, Germany). They were used without further purification. The standard mixture containing the 60 VOCs of EPA method 502.2 was obtained from Alltech (Deerfield, IL, USA). Methanol (Baker, Instra-analysed, Phillipsburg, NJ, USA) was used as solvent for the preparation of standard solutions. 1,1,1-Trifluorotoluene (Aldrich, Milwaukee, WI, USA) was used as internal standard (IS). Vocarb 4000 traps (8.5 cm Carbopack C, 10 cm Carbopack B, 6 cm Carboxen 1000 and 1 cm Carboxen 1001) were obtained from Supelco (Bellefonte, WI, USA) and used as adsorption traps (1/8" OD). Water used for the preparation of blanks and standards was obtained from Baker and 1-octanol used for the reduction of sample foaming was obtained from Merck.

Equipment

A microprocessor-controlled P&T system, the Tekmar LSC-2000 (Tekmar, Cincinnati, OH, USA), was coupled to a GC-MS (Finnigan Magnum Ion Trap MS, Finnigan, San José, CA, USA) via a heated transfer line terminating in a cryogenic focuser at the GC end. The internal lines of the P&T are constructed from glass-lined stainless steel, and the transfer line and internal lines are connected via a heated 6-port switch valve. The standard needle sparger of the Tekmar was replaced with a system consisting of two needles (purge gas in- and outlet) and a moisture trap, which was a 40-ml vial cooled to –10 °C (Figure 2.2.1). The 40-ml open whole screw cap vials (moisture trap and sample vials) and PTFE/silicone liners were obtained from Alltech.

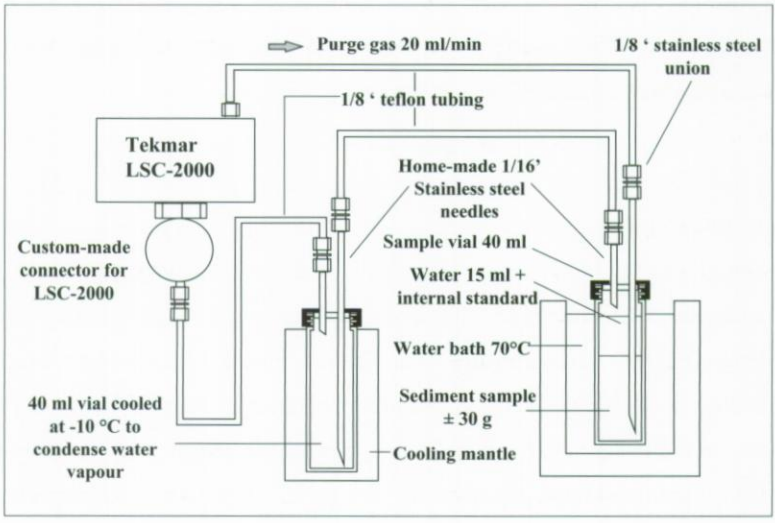

Figure 2.2.1: On-line P&T set-up

Analytical procedure

Preparation of blanks Water specially prepared for the analysis of VOCs (Baker) was used to prepare blanks and standard solutions (see below). Water was continuously purged during storage with nitrogen. For the preparation of blank samples 1 µl of the internal standard was added to 25 ml of the treated water which was then treated as a sample.

Preparation of standard solutions A more detailed description of the preparation of standard solutions is given elsewhere [9]. For calibration of the procedure, 1 µl of a methanolic solution containing 0.4-0.8 ng/µl of the various target compounds and 1 µl of a methanolic solution containing the internal standard (about 0.4 ng/µl) were added to 25 ml of blank water (see above). The water was then injected into a 40-ml sample vial, and the sample vial connected to the on-line P&T set-up, pre-concentrated and analysed by GC-MS. The procedure for spiked samples was identical but had an additional settling period of 24 hours.

Samples, Sample pre-treatment and analysis

Eel, with a length between 20 and 40 cm, were collected in the industrial zone of the Scheldt estuary near Antwerp. Approximately 15 g of muscle tissue from each eel was

homogenised with an Ultra-Turrax blender at 0°C and transferred to a 40-ml sample vial. After the addition of 25 ml organic-free water, 1 µl of the internal standard (1,1,1-trifluorotoluene) solution and 20 µl of 1-octanol, the vial was closed with an open hole screw cap with a PFTE-silicone rubber septum and the homogenate treated in a ultrasonic bath (20 min at 0°C) to further disrupt the tissue. The glass vessel was then coupled to the P&T system by puncturing the septum with the two needles. The volatiles were forced out of the sample by purging the sample for 34 min with a 20 ml/min stream of helium at 70°C (water bath). The analytes were trapped onto a Vocarb 4000 sorbent trap mounted in the P&T apparatus at a temperature of 45 °C. After purging, the trap was backflushed while being rapidly heated to 250 °C and the analytes were desorbed into a cryofocusing module connected to the analytical column. The cryofocusing module was either cooled to –120 °C, for an analysis involving cryofocusing, or kept at a constant temperature of 250 °C for an analysis without cryofocusing.

The analytes were injected into the GC column by rapidly heating the cryofocusing module from –120°C to 200 °C in 0.75 min or by direct transfer, *i.e.* without cryofocusing, to the GC column. Separation was done on a 60 m x 0.25 mm ID J&W DB-VRX column with a film thickness of 1.4 µm. Temperature programming of the GC and data acquisition were started simultaneously. The temperature of the GC oven was held at 35 °C for 6 min and then linearly increased from 35 °C to 200 °C at 4 °C/min, and finally held at 200 °C for 4 min. Helium was used as the carrier gas with an inlet pressure of 16 psi. The target compounds were identified on the basis of their retention times and mass spectra and quantified using the total mass of selected ions (see Table 2.2.1 below). The ion trap detector was operated in the electron ionisation (EI) mode with the multiplier voltage set at 2400 V, the axial modulation (A/M) amplitude at 3.5 V and the emission current at 12 µA. The manifold temperature was set at 220 ° C. The mass range was 50-250 amu and the scan rate 1000 ms. The filament delay was 180 s, and a mass defect of 50 mmass / 100 amu and a background mass of 55 amu were selected.

Analytical quality assurance

A detailed description of the analytical quality assurance is given elsewhere [9]. Blanks were run with each series of samples and compared with previously recorded blanks and the standard solution. Further measures included monitoring the response factors of the standards and treating a standard solution as a sample.

Table 2.2.1: Sequence number, selected ion masses, retention time and LOD (for 40-g sample) for the VOC mixture determined in the eel samples.

Compound	Sequence number	Selected* masses	Retention time (min)	LOD for (pg/g)
Trichlorofluoromethane	1	101/103/66	4:13	40
1,1-Dichloroethene	2	61/63/96	4:59	6
Methylene chloride	3	84/86/49	5:56	9
trans-1,2-Dichloroethene	4	61/96/98	6:26	7
1,1-Dichloroethane	5	63/83/97	6:58	6
cis 1,2-Dichloroethene	6	61/96/98	8:18	5
2,2-Dichloroprane	7	77/79/97	9:03	7
Bromochloromethane	8	130/128/49	8:42	10
Chloroform	9	83/85	8:53	3
1,1,1-Trichloroethane	10	97/61/99	11:15	8
Tetrachloromethane	11	117/119	12:40	4
Dichloropropene	12	39/110/77	12:04	10
Benzene	13	78	12:57	1
1,2-Dichloroethane	14	62/64	10:57	3
Trichloroethene	15	130/95/60	16:28	40
1,2-dichloropropane	16	62/63/76	16:08	20
Dibromomethane	17	174/172/93	15:47	20
Bromodichloromethane	18	83/85/47	16:41	30
Trifluorotoluene	IS	146/127/96	18:24	na
cis-1,3-Dichloropropene	19	75/110/39	20:27	2
Toluene	20	91	24:04	1
trans-1,3-Dichloropropene	21	75/110/39	22:45	2
1,1,2-Trichloroethane	22	97/61/99	23:13	10
Tetrachloroethene	23	166/129/94	27:08	2
1,3-Tichloropropane	24	76/78/41	24:23	9
Dibromochloromethane	25	129/127/48	25:12	2
1,2-Dibromoethane	26	107/109/27	26:11	3
Chlorobenzene	27	112/114/77	29:58	1
1,1,1,2-Tetrachloroethane	28	131/133/95/122	29:42	3
Ethylbenzene	29	91/105/106	31:03	2
m-Xylene	30	91/105/106	31:55	1
p-Xylene	31	91/105/106	31:55	1
o-Xylene	32	91/105/106	33:24	2
Styrene	33	103/78/51	33:08	2
Bromoform	34	173/171/175	34:13	3
Isopropylbenzene	35	105/125/77	34:54	1
1,1,2,2-Tetrachloroethane	36	83/85/131/133	33:21	6
Bromobenzene	37	158/156/77	35:25	1
1,2,3-Trichloropropane	38	75/110/39	33:53	20
n-Propylbenzene	39	91/100/125	36:35	2
2-Chlorotoluene	40	91/126	36:44	4
1,3,5-Trimethylbenzene	41	105/125/77	37:55	4
4-Chlorotoluene	42	91/126	37:03	6
tert.-Butylbenzene	43	91/119	38:45	3
1,2,4-Trimethylbenzene	44	77/105/125	39:18	5
sec.-Butylbenzene	45	134/105	39:36	20
1,3-Dichlorobenzene	46	146/111/75	39:34	10
p-Isopropyltoluene	47	119/91/39	40:26	10
1,4-Dichlorobenzene	48	148/146/111/75	39:52	10
1,2-Dichlorobenzene	50	146/111/75	41:06	2
1,2-Dibromo-3-chloropropane	51	157/75/57	43:24	4
1,2,4-Trichlorobenzene	52	180/145/109	46:59:00	90
Hexachlorobutadiene	53	260/225/190	49:14:00	2
Naphthalene	54	128/102	48:46:00	3
1,2,3-Trichlorobenzene	55	180/145/109	49:29:00	9

na = not applicable (IS), * In order of relative abundance

2.2.3 Results and discussion

Analytical data and methodology

The first major modification of our previous P&T procedure [9] was the elimination of the spargers with Wheaton connectors in favour of the system presented in Figure 2.2.1. The spargers were prone to leaking after a period of intensive use, because the PTFE liner of the Wheaton connector deformed at the temperatures and pressure used. Sample vials are now connected to the system simply by puncturing the septa. This connection virtually eliminated the occurrence of leaks during purging. Also, there is no longer any need to open the sample vials in order to connect them to the on-line P&T, which essentially prevents losses due to volatilisation of the analytes and contamination by laboratory air. The latter is a well known problem in the field of VOC analysis and was thoroughly discussed in our previous work [9]. During this work, both the blank levels, which ranged from 1 to 90 pg/ml, and their variability, which varied between 10 and 120%, were similar to the earlier reported results. In the light of these and previous results contamination during homogenisation and equipment background are still considered to be primary causes of the observed blank levels. The new set-up also uses larger vials, which permit a larger sample intake and, consequently, improve analyte delectability. An additional benefit of the larger vial is the possibility to homogenise the biological tissue in the vial itself, which keeps the sample handling, with all its associated dangers, to a minimum.

In earlier studies, sample foaming caused some problems when biological tissues were purged at elevated temperatures [9,18,19]. Contrary to what is reported by Michael et al. [18], addition of 1-octanol totally eliminated sample foaming and allowed an increase of the purge flow to 20 ml/min. A higher flow was impossible due to technical restrictions of the Tekmar P&T, but in an off-line set-up no sample foaming was observed at flows of up to 40 ml/min. Such a higher flow would certainly further decrease the purging time by at least 50% and, consequently, reduce the overall analysis time. This aspect needs to be further investigated.

As there were practically no changes in the operational parameters of the original set-up only the purge time for the new volume of 40 ml and purge flow of 20 ml/min was evaluated. Since the two-fold increase in the sample volume was compensated by the twofold increase in purge flow, it was assumed that the original purge flow could be

maintained. This was confirmed by a recovery test for those VOCs that are considered priority hazardous compounds [2-4]. The results are given in Table 2.2.2. The recoveries were better than 80% for all analytes except 1,1-dichloroethane (59%), which is the most volatile member of the group. These results are fully satisfactory when compared with the recoveries reported in the literature, which vary from 40 to 130% [11-14,16]. With the original method, the recoveries were between 63 and 115%, however, the variability of the recovery data then was higher. This indicates the increased robustness of the current set-up, a conclusion which is confirmed by the precision data now obtained (Table 2). For ten out of twelve test analytes, the RSD values were 14-17% whereas previously reported repeatabilities varied between 5 and 30% [9,11-14,16]. In summary, the analytical data for the test set are of good quality.

Table 2.2.2: Recovery and repeatability data for the target compounds*.

Compound	Concentration (ng/g)	Recovery (%) n=5	RSD (%; n=5)
1,1-Dichloroethane	0.49	59	17
Chloroform	0.45	88	16
Trichloroethane	0.52	97	17
Tetrachloromethane	0.29	99	17
1,2-Dichloroethane	0.49	97	23
Benzene	0.29	92	4.3
Trichloroethene	0.54	95	16
Toluene	0.29	86	17
Tetrachloroethene	0.58	92	15
Ethylbenzene	0.29	82	14
m&p-Xylene	0.42	82	14
o-Xylene	0.39	81	14

* P&T-GC-MS analysis of spiked sample

Extending the application range

For an exploration of the feasibility of analysing a larger number of VOCs and to determine the separation power of the analytical column, a standard mixture of VOCs had to be chosen that would cover a large number of VOCs with mutually similar physicochemical properties. To this end, the standard mixture of 60 VOCs specified in EPA method 502.2 was selected. Method 502.2 is routinely used for the determination of a large number of volatile organic compounds in drinking water by P&T. A typical GC-MS trace of the standard mixture at the concentrations used in this study is shown in Fig.

2.2.2. The current set-up is seen to allow the separation of most VOCs with the exception of m- and p-xylene, o-xylene and 1,1,2,2-tetrachloroethane and sec.-butylbenzene and 1,3-dichlorobenzene. However, o-xylene and 1,1,2,2-tetrachloroethane have totally different mass spectra and the sum of their most prominent peaks (m/z 83,85,131,133 and m/z 91,105,106 respectively) can be used for quantification, while sec.-butylbenzene can be distinguished from 1,3-dichlorobenzene on the basis of m/z 146, 148, 75, 109. Or, in other words, only the m-xylene–plus–p-xylene pair could not be distinguished even when applying selected ion monitoring.

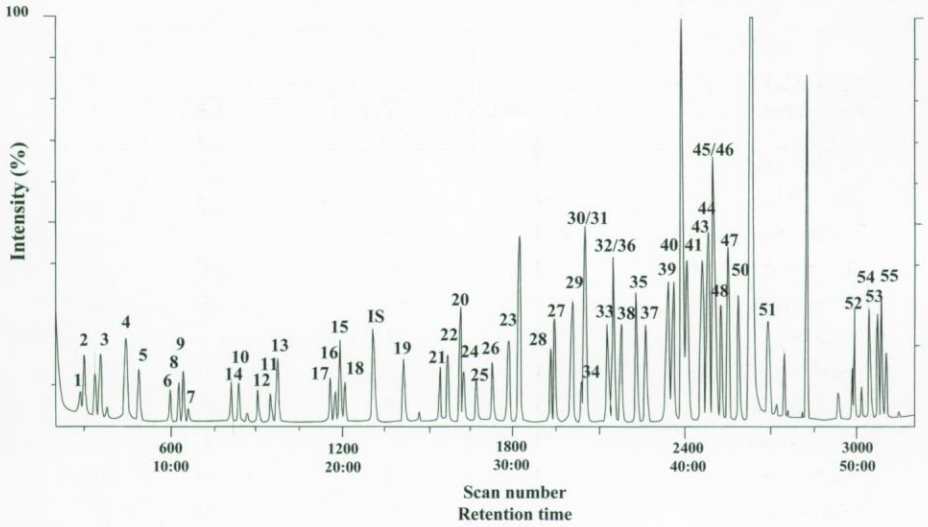

Figure 2.2.2: Full-scan GC-MS separation of 56 VOCs on a 60 m x 0.25 mm ID J&W DB-VRX column (film 1.4 µm). For details, see Experimental.

The use of the long DB-VRX column requires a high inlet pressure. With the normal inlet pressure of 24 psi, a shift of the ion masses with one mass unit was noted and attributed to an insufficient amount of He entering the ion trap. Increasing the inlet pressure to 28 psi indeed solved the problem. The most prominent feature of using the DB-VRX column is that it allows analysis without cryofocusing. This was tested by desorbing the analytes from the trap directly into the analytical column while the cryofocusing module was kept at 250 °C. Figure 2.2.3 clearly shows that eliminating the cryofocusing step has no

influence on the separation, as peak shapes and retention times were not altered at all. Due to a combination of column dimensions, film thickness and oven temperature the analytes were sufficiently focused at the beginning of the column, which makes cryofocusing superfluous. This simplification further improves the robustness of the method, because a constant supply of liquid nitrogen is no longer required. With the previous set-up, the liquid nitrogen supply occasionally became depleted during a run, which resulted in the loss of time as well as sample.

Table 2.2.3: VOC detected in eel from the Scheldt estuary and laboratory blanks.

Compound		VOC levels (ng/g fresh weight) in:			Blank level (ng/g)
Sequence number	Name	Eel 1	Eel 2	Eel 3	
1	Trichlorofluoromethane	170	396	42	nd
2	1,1-Dichloroethene	nd	nd	15	nd
3	Methylene chloride	8.3	nd	nd	nd
9	Chloroform	82	12	4.0	0.15
14	1,2-Dichloroethane	0.39	0.35	0.17	0.004
10	1,1,1-Trichloroethane	1.6	2.1	1.2	0.005
11	Tetrachloromethane	1.0	1.1	nd	0.006
13	Benzene	2.0	2.4	1.2	0.09
17	Dibromomethane	1.5	1.1	0.74	nd
15	Trichloroethene	6.5	7.7	5.1	nd
22	1,1,2-Trichloroethane	0.53	nd	nd	nd
20	Toluene	3.8	3.2	1.3	0.06
25	Dibromochloromethane	0.29	0.17	0.09	0.003
23	Tetrachloroethene	15	16	11	0.06
27	Chlorobenzene	0.34	0.41	0.24	0.01
29	Ethylbenzene	0.71	0.71	0.40	0.04
34	Bromoform	1.2	0.70	0.60	nd
30/31	m-Xylene & p-xylene	0.92	0.74	0.41	0.03
33	Styrene	1.2	0.54	0.37	nd
32	o-Xylene	1.1	0.99	0.65	0.02
35	Isopropylbenzene	0.42	0.56	0.36	nd
37	Bromobenzene	0.16	0.13	nd	0.008
40	4-Chlorotoluene	nd	nd	1.3	nd
41	1,2,4-Trimethylbenzene	nd	0.33	nd	0.03
46	1,3-Dichlorobenzene	1.6	nd	0.37	0.06
48	1,4-Dichlorobenzene	3.3	0.82	1.7	0.05
50	1,2-Dichlorobenzene	1.6	0.41	0.83	0.03

nd = below detection limit

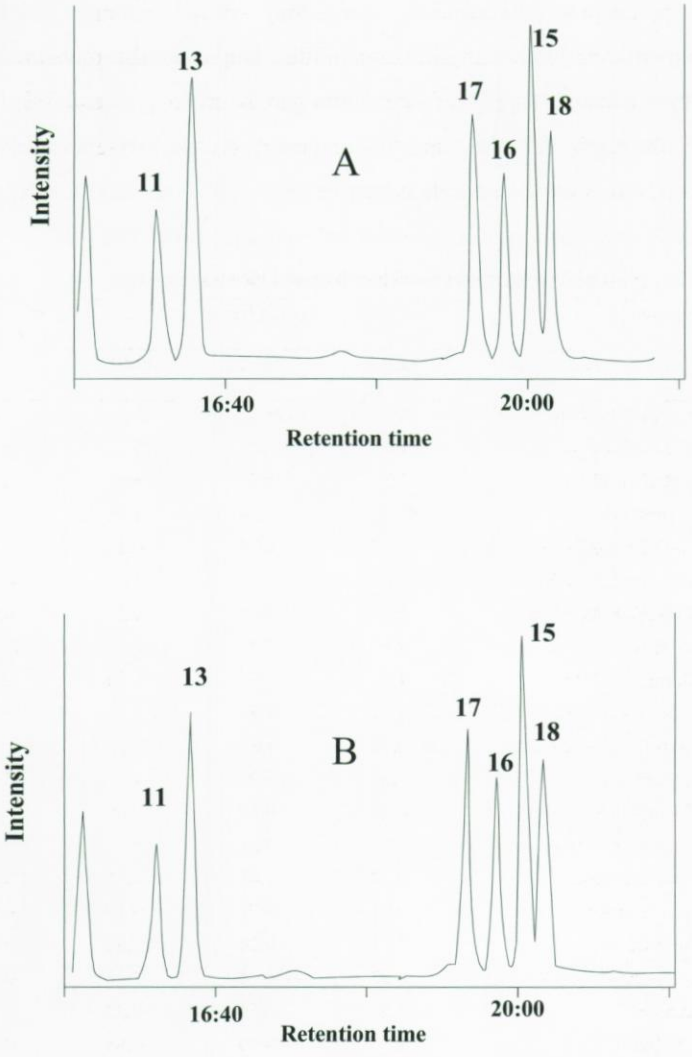

Figure 2.2.3: Effect of eliminating cryofocusing on performance. (A) With cryofocusing, (B) without cryofocusing. For peak number indentification see Table 2.

The combined results obtained so far indicate that 54 out of the 60 VOCs routinely analysed in water with EPA method 502.2, can be determined in biological tissue using the present procedure. Exceptions are the most volatile compounds, dichlorodifluoromethane, chloromethane, vinylchloride, bromomethane and chloroethane, with boiling points in the –29 to 12 °C range, and n-butylbenzene. Although insufficiency of the present procedure for the volatile compounds is probably a result of the methodology used, this is not the case for n-butylbenzene. The latter analyte coelutes with an interfering peak that was found to be invariably present in our P&T-GC-MS system and cannot be distinguished from it even with selected ion monitoring.

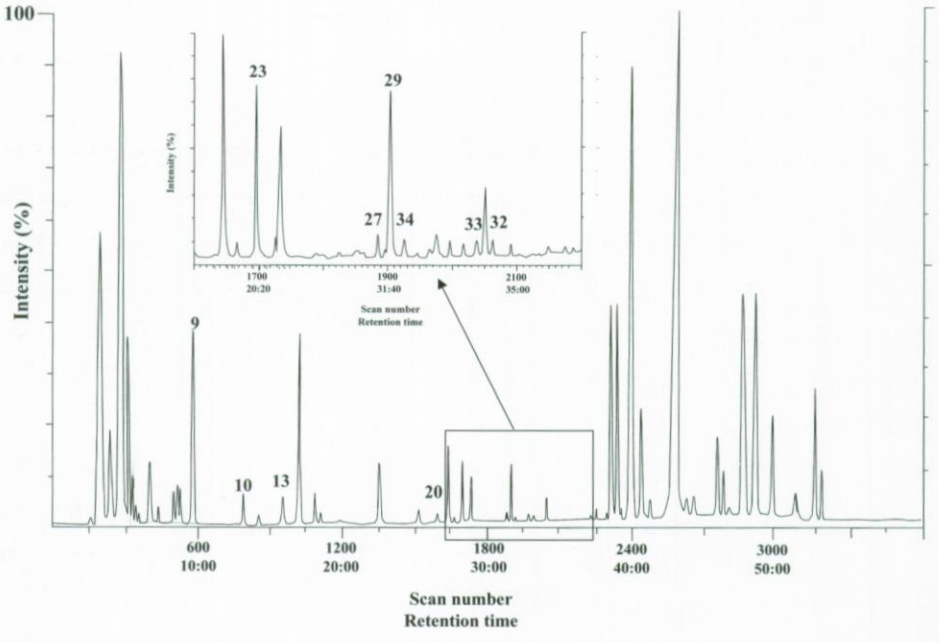

Figure 2.2.4: GC-MS chromatogram of eel no. 1 (cf. Table 3) from the Scheldt estuary (box enlarged as insert). For conditions, see Experimental.

Analysis of eel samples

Three eel samples from the Scheldt estuary were used to test the practicality of the present approach. Compounds were identified on the basis of their mass spectra and their concentrations were calculated on the basis of selected ion masses. Limits of detection (LODs) were calculated on the basis of a signal-to-noise ratio of 3 or the blank + 3 sd. All relevant data are presented in Table 2.2.2. The results show that all target compounds of Table 2.2.1 except 1,1-dichloroethane were present (Table 2.2.3). Although the majority of the other VOCs (i.e. 15 out of 44) was not detected in any of the samples, several additional VOCs were found such as, e.g. trichlorofluoromethane, brominated methanes, styrene and chlorinated benzenes (Table 2.2.3).

As an illustration, a GC-MS trace for eel sample No. 1 is shown in Fig. 2.2.4. The most striking observation is the occurrence of trichlorofluoromethane at concentrations of 40-400 ng/g fresh weight, especially since the compound was not detected in the blank so that contamination cannot have played a role. Trichlorofluoromethane or Freon 11 was primarily released to the environment when it was used as an aerosol propellant. Other sources of emission include its use as a refrigerant, foaming agent, solvent and degreaser [1]. The bioconcentration potential of trichlorofluoromethane is assumed to be negligible [1]. Dickson and Riley [6] reported concentrations of trichlorofluoromethane of 0.1-5 ng/g on a dry weight basis in various marine organisms and 2-20-fold enrichment compared to the water column. The concentrations reported here for eel are much higher, which certainly raises questions about the exposure of the eel to this compound. The brominated compounds found in the eel may well be linked to inadvertent formation during chlorination of drinking water [20]. Helz and Hsu [21] defined transfer from the atmosphere, in situ biosynthesis, in situ chemical synthesis and industrial or municipal waste discharge as the four main ways in which volatile halocarbons are introduced into coastal waters. In this case the latter can be expected to be the predominant source. The presence of chlorinated benzenes is probably due to the various industrial processes in and around the harbour area. Howard [1] quotes concentrations reported by several authors for fish and seafood, which are generally in the low ng/g range on a fresh weight basis. The present results are of the same order of magnitude. Styrene emissions are typically caused by spillage during production and/or use; styrene is also present in automobile exhausts [1]. However, although the compound is one of the most widely used

raw materials in the polymer industry [22], concentrations comparable to those in Table 2.2.3 have not been reported in the literature.

Finally, the average VOC concentrations in eel were compared with the average concentrations in sediment, determined by using the same procedure, and average concentrations for water, covering a period of two years, which were reported by Dewulf et al. [23]. Figure 2.2.5 shows that the concentrations of the target VOCs are several orders of magnitude higher in eel, which again raises questions about the potential to bioconcentrate VOCs and the exposure of fish to these. All compounds discussed during this study are considered to have a low tendency to be bioconcentrated and are therefore not regarded as a potential threat to organisms. Yet during the present and a previous study [9], VOC concentrations occasionally were much higher than what is expected on the basis of their bioconcentration factor (BCF). An overview of calculated and reported BCFs is given in Table 2.2.4. The BCF for chloroform, for instance, is 6 [24], or in other words, concentrations in the organism should be some 6-fold higher than concentrations in the water. Yet the data of Table 2.2.4 show an approx. 100-fold difference. Similarly, the BCFs of tetrachloromethane and toluene calculated from our data are 40-fold and 30-fold higher, respectively, than published BCF data. For the other VOCs the discrepancy between published and calculated BCFs is smaller, i.e. 2-10-fold. Moreover, one should consider that for most of the VOCs in Fig. 2.2.5, the concentration levels are comparable

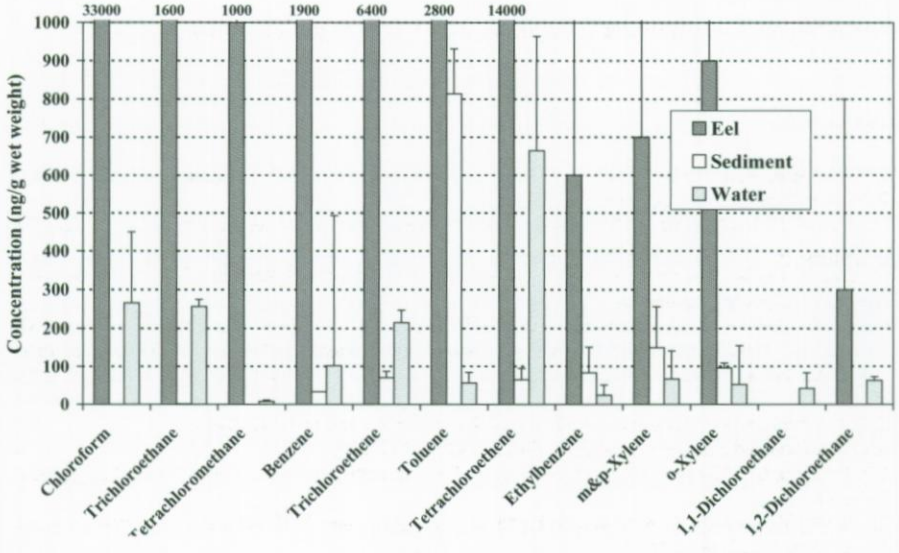

Figure 2.2.5: Comparison of VOC the concentrations in water, sediment and eel from the same area.

to those of well-known contaminants such as individual CB congeners [25]. The observed levels will probably not cause acute toxic effects, and therefore pose no immediate threat to organisms. The danger lies in the continuous, i.e. long-term, exposure of organisms to low levels of contaminants [21]. Actually, several compounds detected in the organisms are either proven or suspected carcinogens [26].

Table 2.2.4 Comparison between calculated (BCF_{Calc}) and reported bioconcentration factors (BCF_{Lit}).

Compound	BCF_{Calc}	BCF_{Lit} *
Chloroform	620	6.0
Trichloroethane	30	8.9
Tetrachloromethane	640	17
Benzene	95	13
Trichloroethene	150	17
Toluene	250	8.3
Tetrachloroethene	105	49
Ethylbenzene	125	15
m&p-Xylene	50	15
o-Xylene	85	21
1,2-Dichloroethane	25	2.0

* Data from references [1,10,24]

2.2.4 Conclusion

To conclude, the environmental significance of low levels of VOCs in organisms deserves further attention. The present analytical methodology of P&T combined on-line with GC-MS can significantly contribute in this field because it provides a robust, sensitive and highly selective way to determine a large range of VOCs in biological tissues.

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2.3 Evaluating the use of a high-resolution time-of-flight mass spectrometer for the determination of selected environmental contaminants[§]

Summary

A benchtop high-resolution time-of-flight mass spectrometer (TOF MS) was evaluated for the determination of key organic microcontaminants. The major advantage of the TOF MS proved to be the high mass resolution of about 0.002 Da (10 ppm). Consequently, the detectability of polar pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls is excellent, and detection limits are in the order of 1–4 pg injected mass. Best mass spectral resolution was obtained for medium-scale peaks. It is a disadvantage that the calibration range is rather limited, *viz.* to about two orders of magnitude. The high mass spectral resolution was especially useful to improve the selectivity and sensitivity when analyzing target compounds in complex samples and to prevent false-positive identifications.

[§] From *J. Chromatogr. A*, 970 (2002) 213-223, also published in J. Dallüge, PhD thesis, Free University, Amsterdam, the Netherlands, 2003.

2.3.1 Introduction

Today, three types of commercially available mass spectrometers (MS) are mainly used in combination with gas chromatography (GC), quadrupole, ion-trap, and sector instruments. With the introduction of relatively inexpensive and user-friendly benchtop quadrupole and ion-trap instruments, MS detection became available for routine operation in GC. Both types of instrument provide unit mass resolution (R<1000), moderate scan speeds of up to 10 spectra/s and detection limits in the low-pg range. Sector instruments provide a much higher mass resolution (R>10,000). Usually, they are operated in the selected-ion monitoring (SIM) mode or used to scan over a narrow mass range, and are used for the target analysis of, *e.g.*, polychlorinated dibenzodioxins and -furans [2], biphenyls (CBs) [3] or toxaphene [4]. Sector instruments trade sensitivity for resolution – the higher mass resolution is obtained by using narrow slits, which allows only ions in a narrow *m/z* range only to pass through [1]. Detectability is similar to that of quadrupole and ion-trap detectors in the SIM mode at a much higher mass resolution; however, operated in the full-scan mode, the scan speed then is typically 3 scans/s. In addition, they are expensive and bulky, and experienced operators are required.

Some seven years ago, the first commercially available time-of-flight mass spectrometer (TOF MS) was introduced for analytical purposes. In contrast to the above MS systems, which use an electrical or magnetic field to separate ions with different *m/z* values, TOF MS instruments measure the time an ion needs to travel through a field-free region. The ions generated in the ion source, are accelerated as discrete packages into the field-free flight tube by using a pulsed electrical field. Flight times – which are proportional to the square root of the *m/z* of an ion – are in the order of microseconds. Consequently, TOF MS can be operated at very high repetition rates, typically 5–30 kHz, *i.e.* 5000–30,000 raw mass spectra are generated per second. Of course, fast detector electronics (which were not available or too expensive until a few years ago) are required to record the arrival times of the ions at the end of the flight tube. A number of the raw mass spectra are added or averaged and, typically, 10–500 spectra/s are stored in the computer system. [5,6,7]

The fast scan speed makes TOF MS very suitable for fast, flash or comprehensive GC. In addition, because discrete packages of ions are analyzed in the flight tube, analyte concentrations do not change during the 'scan' of one raw mass spectrum; consequently,

TOF MS is not prone to skewing. Due to the high repetition rate, a large fraction of the ions generated in the ion source is pulsed into the flight tube, and during separation in that tube, no ions are lost (which does occur with scanning instruments such as the quadrupole MS). Consequently the duty cycle of a TOF MS is 20–30% as against 0.1–1% for a scanning instrument. As a result, sensitivity will be higher for TOF MS, than for the other instruments when operated in the *scanning* mode.

In TOF MS, there are today two more or less complementary approaches, with instruments that provide high resolution (5–10 ppm) [7] but have a moderate scan speed (ca. 10 Hz), and instruments that feature a high storage speed of, typically, 100–500 spectra/s but usually provide only unit-mass resolution (or, as actually should be said, a resolution of 300–1500; at 50% peak-height definition). In the past few years, high-speed instruments have repeatedly been used successfully as detectors of choice for fast and comprehensive GC [6, 8]. The LECO (St. Joseph, MI, USA) TOF MS Model Pegasus II is the instrument used in most of these studies. Recently, a benchtop high-resolution mass spectrometer has been marketed by Micromass (Manchester, UK). It is, therefore, of distinct interest to study the capabilities of this instrument for the identification/determination of key organic micropollutants and to briefly compare the merits of both approaches.

2.3.2 Experimental

Materials

All chemicals used were of research-grade quality. Methyl acetate was distilled before use. A standard containing 40 nitrogen- and/or phosphorus-containing pesticides (code NPM-525C), a PCB standard (EPA PCB congener calibration check solution), and a mixture of the 16 EPA PAHs were obtained from J.T. Baker (Deventer, the Netherlands).

Methods

Instrumental. Analyses were performed on a HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) using a GCT time-of-flight mass spectrometer (Micromass, Manchester, UK) as detector. The GCT TOF MS was equipped with a 1 GHz time-to-digital converter. Injections were done in the on-column mode using a 1 m x 0.53 mm I.D. retention gap. Pesticides and PAHs were separated on a 30 m x 0.25 mm

I.D. x 0.25 µm DB-5 MS column (J&W, Folsom, CA, USA); the PCBs were separated on a 40 m x 0.18 mm I.D. x 0.18 µm DB-5 column (J&W).

The GCT TOF MS was operated at a multi-channel plate voltage of 2500 V, a pusher interval of 40 µs (resulting in 25,000 raw spectra per second), and a scan range of *m/z* 50–500. The spectrum storage rate was 2 Hz. 2,4,6-Tris-trifluoromethyl-[1,3,5]triazine was used as internal standard for mass calibration with *m/z* 284.9950 as internal reference mass. During analysis, the internal standard was continuously introduced into the ion source. MassLynx software version 3.4 was used for data processing.

For comparison, a HP 6890 (Agilent Technologies) equipped with an Optic 2 programmable injector (ATAS, Veldhoven, the Netherlands) and a Pegasus II TOF MS (LECO, St. Joseph, MI, USA) was used. The LECO TOF MS allows spectrum storage rates of 1–500 spectra per second at mass-unit resolution. The LECO TOF MS was operated at a spectrum storage rate of 2 Hz, using a mass range of *m/z* 45–500 and a multi-channel plate voltage of –2000 V. With this set-up, 1-µl injections were performed in the cold splitless mode. Separations were carried out on a 30 m x 0.25 mm I.D. x 0.25 µm DB-5 MS column (J&W).

Wastewater. Solid-phase extraction (SPE) of the wastewater samples was performed on a Prospekt automated sample preparation system (Spark Holland, Emmen, the Netherlands). The Prospekt system consists of three six-port valves, an automated cartridge exchanger and a solvent delivery unit including solvent selection valves and an LC pump. The solvent for the desorption of the SPE cartridges was delivered by a Phoenix CU20 syringe pump (Carlo Erba Strumentazione, Milan, Italy).

Samples of influent water from a municipal sewage water treatment plant were first centrifuged and, then, filtered through a 0.45 µm membrane filter (type HA, Millipore, Etten-Leur, the Netherlands). SPE was carried out as described in [9] using 50 ml of wastewater. In the final desorption step the analytes were eluted with 200 µl of methyl acetate. The samples were spiked with the pesticide mixture at levels of 0.05–0.1 µg/l.

Eel samples. PCB extraction was based on total lipid extraction according to Bligh and Dyer [10]. The extracted lipids, which had been used for the determination of the fat

content, were redissolved in hexane, and this solution was cleaned on a 5% deactivated alumina (Merck, Darmstadt, Germany) and, next, a 5% deactivated silica (Merck) column. Prior to the final concentration step, tetrachloronaphthalene (Promochem, Wesel, Germany) was added as an internal standard.

2.3.3 Results and discussion

Mass accuracy: dependence on signal intensity

According to its specifications, the GCT TOF MS equipped with a 1 GHz time-to-digital converter should be able to achieve a mass accuracy of better than 10 ppm (above *m/z* 200) or 0.002 Da (below *m/z* 200), provided that the peak of interest has a 'sufficient intensity'. The lower relative mass accuracy (expressed in ppm) below *m/z* 200 is caused by limitations of the detector electronics, since at these low masses much smaller flight-time differences have to be measured. Actually, a further improvement *viz.* to 5 ppm and 0.001 Da, respectively, is possible with an optional 3.6 GHz time-to-digital converter which was, however, not available to us. In this section, two aspects will be studied: (i) the influence of the signal intensity on the mass accuracy and (ii) the effects of a reduced mass accuracy on the peak shape.

Figure 2.3.1A shows the dependence of the mass accuracy (difference between calculated and measured mass in ppm) on the signal intensity for a set of 40 pesticides in the range *m/z* 200–300. The data points were obtained by examining more than 80 single mass spectra (at 2 Hz, *i.e.* obtained by averaging 12,500 raw spectra to achieve high mass accuracy) that were acquired across several chromatographic peaks. The mass accuracy clearly improves with the signal intensity. At intensities below 300 counts, an accuracy of better than 10 ppm was obtained for only half of the examined spectra. Clearly, a signal intensity of about 2000–3000 counts in a single mass spectrum (at 2 Hz) is required to achieve a mass accuracy of better than 10 ppm for the pesticides. Translating this result into the minimum mass of an analyte that has to be injected, two typical examples may be quoted: a signal intensity of 2000 counts corresponded to an injected mass of 150 pg atrazine (at the *m/z* 215.0938 trace) or 20 pg pyrene (*m/z* 202.0783 trace).

The lower mass accuracy at low mass intensities limited the possibility of using narrow mass windows when generating selected ion chromatograms in trace-level studies or for less intense masses in the spectrum. In addition, the mass determination on the lower

slopes of a chromatographic peak (with their lower intensities) will be less accurate, as is shown in Figure 2.3.1B. Consequently, in some instances, the edges of a peak were 'cut off' when using too narrow mass windows because the masses measured at the edges were outside the mass window. This is demonstrated in Figure 2.3.1C for a 25 ppm and a 10 ppm mass window, with atrazine as an example. Such behaviour will result in an underestimation of the peak area; a broader mass window had, therefore, to be used for quantification at trace levels.

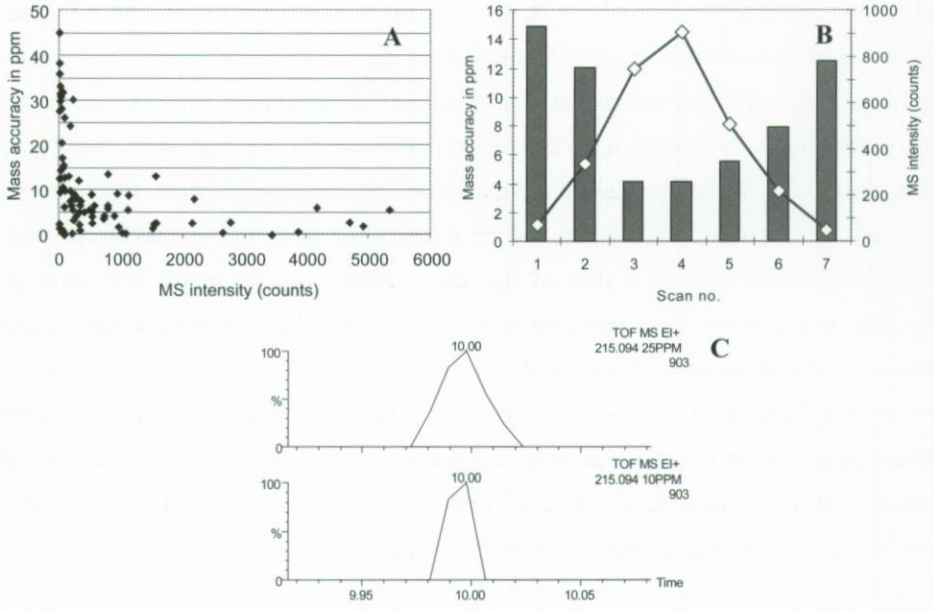

Figure 2.3.1: (A) Dependence of mass accuracy (in ppm) on signal intensity for low concentrations (using 40 test pesticides, mass range m/z 200–300). **(B)** Mass accuracy across a peak with a low intensity. The bars indicate the mass accuracy, the full-drawn line the MS intensity (i.e. peak profile). The example is for a 300 pg injection of atrazine; m/z 215.0938 trace. **(C)** Influence of mass window on peak profile at low concentration. Extract ion chromatogram of atrazine (300 pg); upper trace, 25 ppm mass window; lower trace, 10 ppm mass window.

The lower mass accuracy at these low intensities had, however, little consequence when the accurate mass of a chromatographic peak had to be determined. In that case, usually the mass spectrum at the (intense) peak apex was used. If required, the mass accuracy could be improved by combining (averaging) 3–5 mass spectra across the peak apex, which means that between about 37,000 and 62,000 raw mass spectra were actually

combined. The combined mass spectra feature enhanced signal-to-noise and improved mass accuracy. The algorithm combines peaks in the mass spectrum within a selected mass window (good results were obtained with a window of 0.01 Da) into a single peak. To quote an example, with analytes for which the mass accuracy obtained at the peak maximum was as poor as 28 ppm, the operation effected a distinct improvement to better than 10 ppm.

The data acquisition of the GCT uses a so-called time-to-digital converter (TDC). The TDC is an ion counting system, which can only record the arrival of one ion at a time. After recording such an event, the TDC requires a certain dead time to recover before it can register another ion. At relatively high analyte concentrations and, consequently, higher ion currents it is more likely that one or more ions will arrive within the dead time and, therefore, will not be registered. Quantification will then be incorrect, and the number of non-detected ions will be higher in the higher-*m/z* part of a peak in the mass spectrum. This will cause a shift of the apex towards a lower mass. The software automatically corrects for these effects via a so-called dead-time correction model. However, this dead-time correction does not work at high peak intensities, and the software indicates peaks in the mass spectra that are too intense to use the correction model by a question mark. In our study, this was observed for all peaks that exceeded an intensity of about 6000 counts. The accurate mass can, then, not be determined with sufficient reliability, *i.e.* the identification potential is affected.

The influence of high signal intensities on the measured mass was studied with phthalate esters (*m/z* 149.0239) as an example, because they were present at very high concentrations in some of the samples. As shown in Figure 2.3.2A, the mass accuracy clearly deteriorated at intensities above approx. 50,000 counts. At these high intensities the measured mass was clearly shifted towards lower values, as explained above. However, in the 6000–50,0000 range a mass accuracy of better than 0.006 Da was still achieved. This suggests that accurate masses can still be obtained in, at least, some cases where the software indicates that the dead-time correction model is exceeded. However, the limited information now available does not yet permit us to draw generally valid conclusions.

As an illustration of the decreased mass accuracy across an intense peak, Figure 2.3.2B shows the peak profile and the corresponding mass accuracy across an intense peak of CB 153 in an eel extract. The marked contrast with Figure 2.3.1B is obvious: mass spectra should now be obtained from slopes of the peak because of the higher mass accuracy there. In order to show what can also happen in such situations, Figure 2.3.2C demonstrates that decreased mass accuracy at the peak apex of a very intense peak may lead to split peaks when using narrow mass windows. Therefore, quantification masses should be chosen such that also at high analyte amounts, they are still within the dead-time model. This may imply that, in some cases, a less intense quantification ion has to be selected.

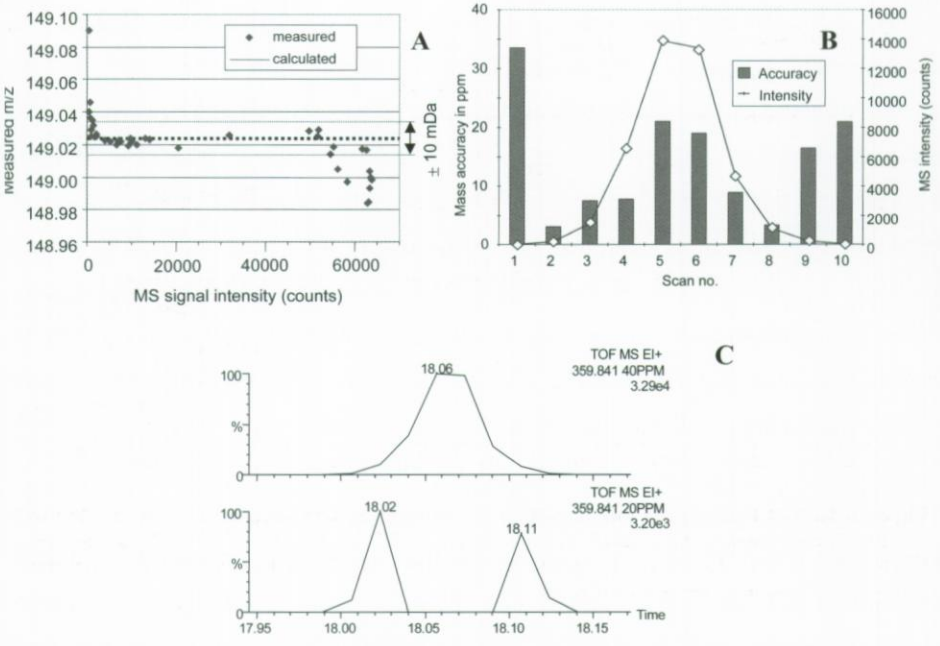

Figure 2.3.2: (A) Dependence of measured mass on signal intensity (using *m/z* 149.0239 mass of phthalates as example). **(B)** Mass accuracy across a peak with a high intensity. The bars indicate the mass accuracy, the full-drawn line the MS intensity (*i.e.* the peak profile). The example is for a 620 pg injection of CB 153 in eel extract; *m/z* 357.8444 trace. **(C)** Influence of mass window on peak profile. Extract ion chromatogram of CB 153 (approx. 620 pg, eel extract); upper trace, 40 ppm mass window; lower trace, 20 ppm mass window.

Due to the continuous introduction of a calibration compound during each analysis, its mass spectrum (containing *m/z* 68.9952, 121.0014, 189.9966, 265.9964 and 284.9949) is superimposed on all other mass spectra. These spectra can, therefore, not directly be used

for library searching; a background subtraction has to be performed prior to the search. As an example, Figure 2.3.3A shows the mass spectrum obtained at the peak apex of CB 66 in an eel extract. The mass of the $^{12}C_{12}H_5{}^{35}Cl_5$ isotope peak at *m/z* 323.8817 was measured with a mass error of 5.3 ppm (theoretical value, *m/z* 323.8834). The combination of four spectra across the peak resulted in an improved mass accuracy with an error of only 1.8 ppm (*m/z* 323.8828, Figure 2.3.3B). During this process, a background subtraction was also performed to remove the interfering masses of the calibration compound at *m/z* 265.9946 and 284.9950 providing a much cleaner spectrum. Figure 2.3.3C shows the calculated isotope peaks for $C_{12}H_5Cl_5$.

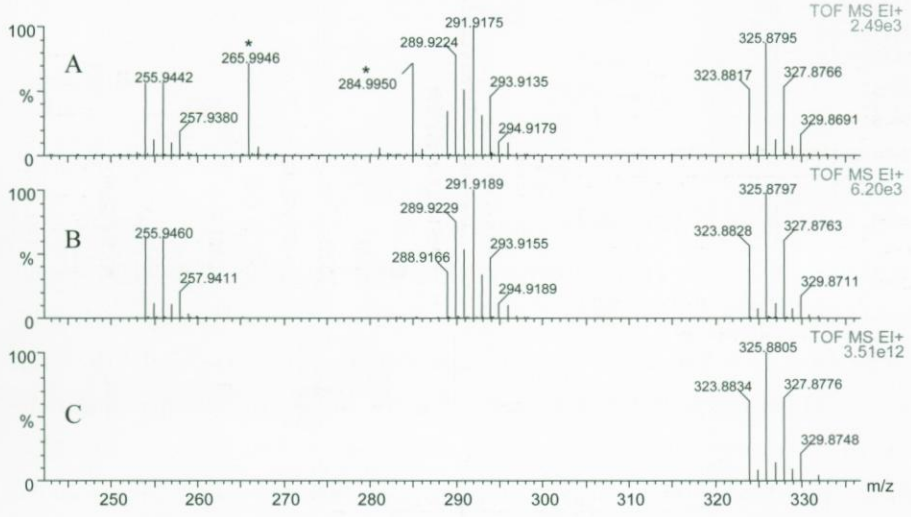

Figure 2.3.3: (A) Part of mass spectrum of CB 66 obtained at peak apex. Peaks indicated with an asterisk (*) are masses of the calibration compound (*m/z* 265.9946 and 284.9950). **(B)** Mass spectrum obtained by averaging four spectra across the peak and subtracting the background. **(C)** Mass spectrum generated for isotope cluster of $C_{12}H_5Cl_5$.

One final remark should be made here. With many complex biological and environmental samples, peak overlap occurs throughout the chromatogram and the recorded mass spectra are, consequently, impure. This causes no insurmountable problems when target analysis performed. If, however, non-target analysis is a relevant aspect of the study, then the automated resolution of the mass spectra of co-eluting compounds, *i.e.* obtaining pure spectra by using a deconvolution algorithm, is extremely powerful – much more than manual subtraction. Unfortunately, this option – which is available on the LECO Pegasus

and the Thermo-Finnigan Tempus – is, as yet, not part of the data-processing software of the GCT.

Table 2.3.1: LODs (pg) of selected analytes using accurate-mass (GCT) or unit-mass (GCT, LECO) resolution*

Compound	Quantification mass (*m/z*)	GCT at 0.05 Da	GCT at 1 Da	LECO at 1 Da
Chlorpyriphos	198.9173	2	10	6
Atrazine	200.0703	1	3	4
Prometryn	241.1361	2	3	4
Trifluralin	306.0702	4	4	5
Metolachlor	162.1283	2	4	2
Chrysene	228.0939	1	2	2
Acenaphthylene	152.0626	1	4	0.5
Benzo[a]pyrene	276.0939	2	5	2.5

*: All experiments at 2 Hz

Detection limits and linearity

Detection limits

The detection limits (LODs) were determined by injecting standard solutions with concentrations of 3 and 10 pg/µl. They were calculated for two different mass windows of 1 Da and 0.05 Da. Data were obtained for 40 pesticides and 16 PAHs; selected data are shown in Table 2.3.1. The results were compared with those from a GC–TOF MS system with a LECO Pegasus II TOF MS, using the same GC column and column dimensions and temperature programme. Both detectors were operated at 2 Hz; however, the LECO Pegasus used a detector (multichannel plate) voltage of 2000 V as against 2500 V for the Micromass GCT. The LECO TOF MS was only used to compare detection limits because the main purpose of the two TOF MS systems used is different (high speed *vs.* high resolution; *cf.* above) and an extended comparison is therefore not appropriate. As for Table 2.3.2, because CB extracts are generally complex mixtures with many closely contiguous or even co-eluting congeners, peak heights are often preferred to peak areas for quantification, and both modes of calculation were used.

When using the GCT TOF MS, the LODs for the pesticides, PAHs and CBs were in the order of 1–14 pg when using a 1 Da mass window. In most cases, a narrower mass window of 0.05 Da provided better detectability since noise was reduced, resulting in an up to 5-fold improved result. In some cases, however, where very selective quantification masses were used (*e.g. m/z* 306.0702 for trifluralin), no improvement could be achieved.

For the CBs, there was no essential difference between peak-height and peak-area based LODs.

Table 2.3.2 : LODs (pg) of selected CBs, peak heights (H) or areas (A) for GCT TOF MS using accurate-mass (at 0.05 Da) or unit-mass (1 Da) resolution.

CB congener	Quantification mass (*m/z*)	LODs at 0.05 Da		LODs at 1 Da	
		H	A	H	A
CB 28	255.9613	1	1	3	7
CB 52	289.9224	1	1	4	6
CB 77	289.9224	1	1	4	3
CB 101	323.8834	1	1	4	6
CB 105	323.8834	1	1	4	4
CB 118	323.8834	1	1	4	3
CB 126	323.8834	1	1	4	3
CB 138	357.8444	1	1	5	4
CB 153	357.8444	1	1	4	4
CB 180	391.8054	1	1	5	9
CB 209	493.6885	4	3	14	25

In summary, with GCT TOF MS, and especially with a 0.05 Da window, analyte detectability is excellent for a wide range of a microcontaminants. As for the CBs, the LODs were at least an order of magnitude better than those found with conventional quadrupole systems operated in the full-scan mode [11]. Of course, for this class of compounds, the ECD still is the most sensitive detector but, in most instances, the much improved selectivity of the GCT TOF MS will far outweigh the loss of sensitivity.

Linearity

The linearity was tested over two or three orders of magnitude in the pg range (injected mass). Representative results for some selected analytes are presented in Table 2.3.3. With the PAHs, linear calibration plots were invariably found, but the plots for the pesticides and CBs were best described by second-order polynomes. Neither we nor the GCT's manufacturers can explain the latter somewhat unexpected result.

The quantification masses for the calibration plots for pesticides and CBs were chosen in such a way that no mass error occurred at the highest concentration level (*cf.* Section 3.1). With the PAHs this was impossible since their mass spectra show only little fragmentation and the (intense) molecular ion had to be used for quantification. A mass error was indicated at the highest injected-mass level of the PAHs (300 pg), indicating that the peaks were too intense to reliably calculate an accurate mass. Naphthalene is

included as an example in Table 2.3.3. However, the data for pyrene and chrysene were kept included in the calibration plot, because in both cases mass accuracy was still better than 10 ppm (at intensities of 6000–9000 counts). Generally speaking, the mass error at higher analyte concentrations limited the linearity to about two orders of magnitude.

Table 3.2.3: Correlation coefficients and calibration equations for selected analytes

Analyte	Quantification mass (*m/z*)	R^2	Calibration equation (y: area, x: concentration)	Concentration range (ng/µl)*
Chlorpyriphos	198.9173	0.9996	$y = 4.4127*10^{-5} x^2 + 0.06204 x$	3–1000
Atrazine	215.0938	0.9954	$y = 5.6522*10^{-5} x^2 + 0.07665 x$	10–1000
Prometryn	199.0984	0.9987	$y = 3.7864*10^{-5} x^2 + 0.04429 x$	10–1000
Trifluralin	306.0702	0.9958	$y = 2.9070*10^{-5} x^2 + 0.03167 x$	10–1000
Metolachlor	238.0999	0.9958	$y = 6.4350*10^{-5} x^2 + 0.06157 x$	3–1000
Naphthalene	128.0626	0.9998	$y = 1.2618 x + 3.115$	3 – 100
Pyrene	202.0783	0.9960	$y = 1.1135 x + 7.0067$	3 – 300
Chrysene	228.0939	0.9982	$y = 1.1196 x - 4.2563$	3 – 300
CB 28	255.9613	0.9991	$y = -0.240559 * x^2 + 90.0592 * x - 94.4804$	2 – 200
CB 52	289.9224	0.9953	$y = -0.27345 * x^2 + 75.4055 * x - 158.665$	2 – 200
CB 101	323.8834	0.9995	$y = -0.122038 * x^2 + 36.3936 * x - 29.7716$	2 – 200
CB 118	323.8834	0.9988	$y = -0.137872 * x^2 + 39.6264 * x - 66.7305$	2 – 200
CB 153	357.8444	0.9975	$y = -0.123917 * x^2 + 33.2781 * x - 65.2652$	2 – 200
CB 180	391.8054	0.9991	$y = -0.0959157 * x^2 + 27.1354 * x - 60.1815$	2 – 200
CB 209	493.6885	0.9960	$y = -0.0552169 * x^2 + 14.0631 * x - 31.7155$	2 – 200

*: 5 or 6 data points

Applications

The high mass accuracy is clearly the main advantage of the GCT TOF MS and will be especially useful when analyzing complex samples. Narrower mass windows will provide a better separation of the analytes from co-eluting compounds and will improve the detectability [12]. The two examples of Figure 2.3.4 should serve to illustrate this, with wastewater as the sample type. The spiking level of the extract was 50 pg/µl.

The example of Figures 2.3.4A and B shows that when a mass window of 1 Da (*m/z* 215) was used, atrazine could not be completely separated from a co-eluting compound (Figure 2.3.4A, peak at 10.02 min) and, overall, the baseline was very noisy. However, when a mass window of 0.02 Da was used, the atrazine peak stood out very clearly and most of the noise had disappeared (Figure 2.3.4B); this resulted in a 3-fold improved LOD. The second example is shown in Figure 2.3.4C where the quantification mass, *m/z* 198.9173, of chlorpyriphos in a 1 Da mass window was not selective at all: many peaks show up in the chromatogram and several of these are at least as prominent as chlorpyriphos itself

(eluting at 10.92 min). Narrowing the mass window to 0.02 Da had an effect which is even more dramatic than with the earlier example: in Figure 2.3.4D a prominent analyte peak stands out against an empty background. In this case, the LOD was improved 15–20-fold.

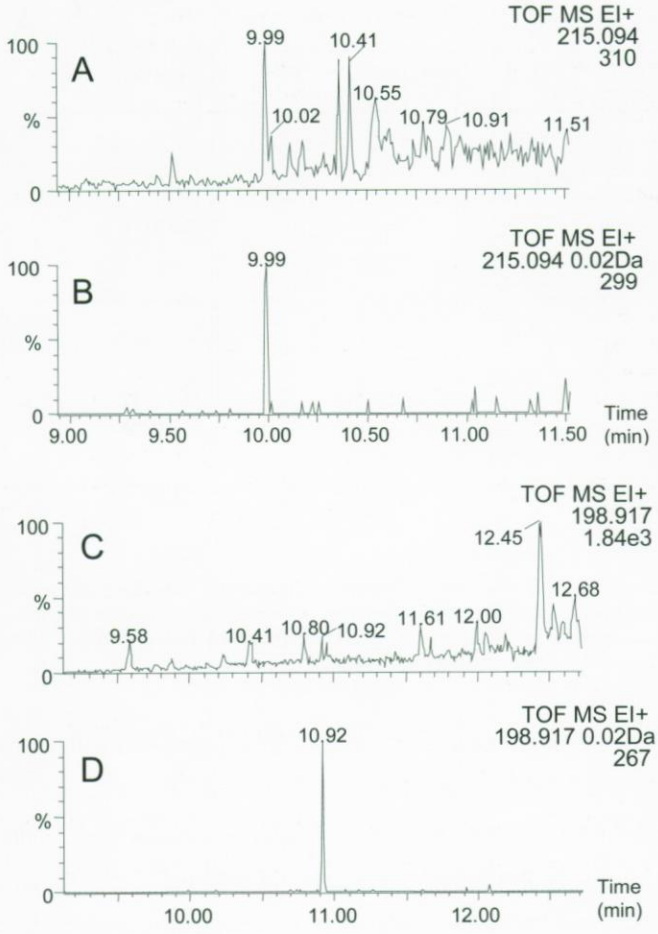

Figure 2.3.4: GCT–TOF MS chromatograms of a wastewater extract (spiked at 50 pg/µl). Extracted ion chromatograms are shown for atrazine (windows A and B; *m/z* 215.0938) and chlorpyriphos (windows C and D; *m/z* 198.9173). The upper chromatograms (A, C) were extracted using a window of 1 Da, the lower chromatograms (B, D) using a mass window of 0.02 Da.

Two further remarks should be made. For the two examples shown, a reduction of the mass window to 0.004 Da did not further improve the S/N ratios because the edges of the chromatographic peaks now were cut off (*cf.* Section 3.1). Secondly, as was earlier observed for trifluralin, using a narrower mass window did not always enhance the

detectability. When, for example, metolachlor was added to the same wastewater sample, the LODs were the same, *viz.* 15 pg, with a 1 Da and a 0.02 Da window. This can be explained by the high selectivity of the *m/z* 162 quantification mass.

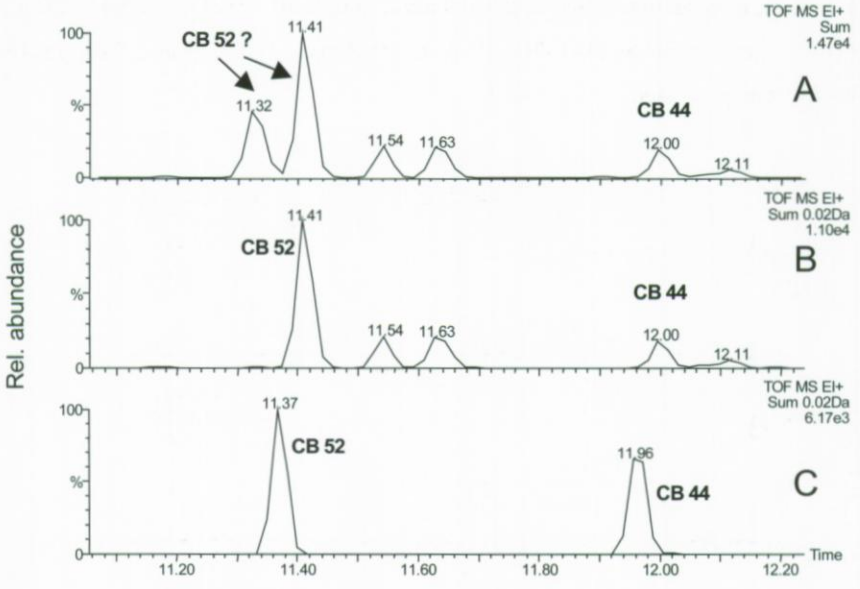

Figure 2.3.5: Influence of the size of the mass windows on the quality of ion chromatograms for an eel sample with high concentrations of CBs. Chromatogram **A** (eel extract) was extracted using a mass window of 1 Da, chromatograms **B** (eel extract) and **C** (standard solution) using 0.02 Da. All chromatograms were extracted using the sum of 10 quantification masses.

LOD calculations for CB in environmental samples were performed with an eel extract that contained only trace levels of these microcontaminants. Even though the general conclusions regarding the GCT were found to hold also in this case, the results differed from those found above for the wastewater in several respects. To quote an example, using a narrower mass window caused only little improvement in analyte detectability, and the LODs (individual data not shown) were 1–2 pg for essentially all CBs, *i.e.* the same as found for standard solutions. Still, the merit of narrow-mass-window recording was clearly shown for contaminants such as, *e.g.*, CB 52, which was present in another eel sample. At nominal mass resolution, no peak was found at the retention time of CB 52 (due to an, initially non-recognized, 0.04-min retention time shift; see Figure 2.3.5C); however, two peaks were found in close proximity (Figure 2.3.5A). Only a narrower mass window revealed that the peak at 11.41 min indeed was CB 52 (Figure 2.3.5B). A similar, but more serious, problem is shown in Figure 2.3.6. In the extracted ion chromatogram of

another extract recorded at a 1 Da window, the fairly large peak eluting at the same time as does CB 118 could easily be mistaken for that compound (Figures 2.3.6A and B, respectively). However, when using an appropriately narrow mass window of 0.02 Da (Figure 2.3.6C), no peak was found at this retention time at all, which means that CB 118 is present in the extract below the LOD of 1 pg injected mass. In this case, a false-positive identification was prevented.

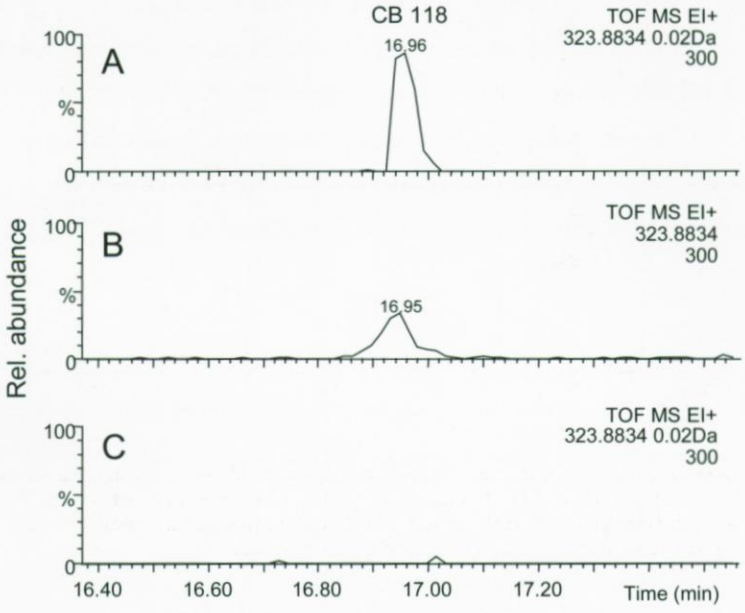

Figure 2.3.6: GCT–TOF MS chromatograms of the *m/z* 323.8834 ion traces of **(A)** a CB standard (10 pg) and **(B, C)** an eel extract. Traces **A** and **C** were extracted by using a window of 0.02 Da, and trace **B** by using a mass window of 1 Da. Signal intensities are the same in all three frames.

2.3.4 Conclusions

The GCT is the first benchtop TOF MS that offers high resolution. The 1 GHz instrument which was tested, achieved a mass accuracy of better than 10 ppm (0.002 Da); at higher analyte amounts or when combining spectra, a mass accuracy of better than 5 ppm was often obtained. The detection limits for pesticides, PAHs and CBs were in the low-pg range. The high mass accuracy allowed the use of narrow mass windows of, typically, 0.02 Da, which substantially improved the identification and quantification of target analytes. However, the accuracy of the measured *m/z* values is

strongly influenced by the signal intensity. It decreases at both too low and too high signal intensities, which is an aspect that has to be considered when selecting the width of the mass windows. This also causes the practically useful ranges of calibration plots to be rather limited.

The high mass resolution of the GCT TOF MS provided excellent selectivity for many of the analytes that were investigated in this study. This selectivity provided improved sensitivity and better identification/confirmation of target compounds in complex matrices. In contrast to sector instruments, the GCT always operates in the scan mode (as do all TOF MS instruments), providing full mass spectra for identification of non-target analytes. One may therefore conclude that the high-resolution GCT instrument (especially once deconvolution software has been developed) can have a role complementary to that of high-speed TOF MS instruments which are to be preferred for fast GC and GCxGC operation.

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3 Baseline monitoring

3.1 Volatile organic compounds in various marine organisms from the southern North Sea**

Summary

The concentrations of 12 priority volatile organic compounds (VOCs) were determined in two species of vertebrates and four species of invertebrates from sampling stations in the Southern North Sea, using a modified Tekmar LSC 2000 purge and trap system coupled to GC-MS. In general, concentrations of VOCs found in this study were of the same order of magnitude as those previously reported in the literature. The concentrations of the chlorinated hydrocarbons (CHCs), with the exception of chloroform, tended to be lower than those of the monocyclic aromatic hydrocarbons (MAHs). The experimental data were statistically evaluated using both cluster and principal component analysis (PCA). From the results of cluster analysis and PCA, no specific groups could be distinguished on the basis of geographical, temporal or biological parameters. However, based on the cluster analysis and the PCA, the VOCs could be divided into three groups, C_2-substituted benzenes, CHCs and benzene plus toluene. This division could be related to different sources. Finally, it was shown that organisms can be used to monitor the presence of VOCs in the marine environment and the observed concentrations levels were compared with proposed safety levels. The results show that, in no case, the safety levels are exceeded.

**From *Mar. Poll. Bull.*, 35 (2001) 1478-1488. 1167 - 1177 40(12)

3.1.1 Introduction

The potential threat of large-production chemicals to the marine environment has caused considerable concern since the deleterious effects of some of these, such as p,p'-DDT, the drins and polychlorinated biphenyls (PCBs), became evident. Much research has since been dedicated to the study of transport mechanisms, environmental distribution, prediction and measurement of fluxes, and adverse environmental effects of important classes of pollutants such as PCBs, aromatic hydrocarbons and heavy metals [1-5]. Much less is known, however, about the fate of more volatile chemicals, even though volatile organic compounds (VOCs) are well-known atmospheric contaminants that are frequently determined in air, drinking water, fresh water, effluents and soils [6-9]. Most representatives of the group are important industrial compounds with a high annual production. The annual production of tetrachloromethane, for instance, is estimated at about 300 000 tonnes [10]. In Belgium, the emissions of the chlorinated hydrocarbons (CHCs) chloroform, 1,1,1-trichloroethane, tri- and tretrachloroethylene, exceed those of e.g. lead, lindane, and atrazine. Annual production, together with log K_{ow} data, toxicity and persistence, was one of the main criteria used by the Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) [11] to select potentially harmful substances for the marine environment. The resulting list contained, amongst others, chloroform, tetrachloromethane, 1,1,1-trichloroethane, trichloroethylene and tetrachloroethylene. The same compounds are also found on the high-priority compounds list of the Ministerial Declarations of the International Conferences of the North Sea [12,13] and are recognised as compounds that present an environmental problem by the Norwegian Pollution Control Authority [14]. For other important VOCs such as the monoaromatic hydrocarbons (MAHs) benzene, toluene, ethylbenzene and the xylenes, the need to investigate their presence in the marine environment has been formulated in the Ministerial Declarations of the International Conferences of the North Sea [12,13].

Despite the potential hazards posed by VOCs, relatively little is known about the abundance or presence of these compounds in the marine environment, especially in biota, and their behaviour in the marine ecosystem. In addition, there are no on-going monitoring programmes for VOCs, in contrast to other organic pollutants such as PCBs [15]. Levels reported in the literature are therefore mostly the result of one-off surveys. Pearson and McConnell [16] were among the first to report concentrations of trichloroethylene, tetrachloroethylene, trichloroethane, tetrachloromethane and

chloroform in various marine organisms from sampling locations along the British coast. The results showed that chlorinated VOCs were present at all trophic levels. The concentrations ranged from 0.02 to 180 ng/g wet weight. Since that time, similar concentrations have been reported for different organisms from various marine locations [16-20]. An overview of the reported concentrations for the different trophic levels is given in Table 3.1.1. Recent findings agree with these earlier observations [21]. Generally speaking, the concentrations are of the same order of magnitude as those of other important organic contaminants such as individual PCBs, chlordanes and individual PAHs [22].

Table 3.1.1: Concentrations in ng/g wet weight of VOCs in various marine organisms (10,16-21).

Organisms	**CHCs**					
	$CHCl_3$	**CCl_4**	**DCE**	**TCE**	**TRCE**	**TECE**
Invertebrates	0.02-1040	0.04-114	1-4080	0.03-310	0.05-250	0.05-176
Marine algae					17-236	13-22
Fish	2-851	0.3-209	730-3200	1-26	0.8-479	0.3-176
Seabirds	1.9-65				2.4-29	1.5-39
	MAHs					
	BENZ	**TOL**	**EBEN**	**MPBEN**	**OBEN**	
Shellfish	220-7000	3.4-18	0.8-250	100-360	520	
Fish	700-1000					

$CHCl_3$ = chloroform, CCl_4 = tetrachloromethane, DCE = 1,2-dichloroethane, TCE = 1,1,1-trichloroethane, TRCE = trichloroethylene, TECE = tetrachloroethylene, BENZ = benzene, TOL = toluene, EBEN = ethylbenzene, MPBEN = m&p-xylene, OBEN = o-xylene.

The effects of these levels on organisms are at present unknown. Most VOCs can be considered as narcotic chemicals, i.e. non-electrolyte chemicals that, in the absence of specific effects, have only a minimum of toxicity [23]. However, halogenated aliphatic compounds in general are considered to be potent immunotoxic agents. Suppression of humoral and cellular immunity as well as host resistance to infections has been observed both in laboratory animals and humans [24]. Also, trichloroethylene has been shown to produce tumours in rodents and is a suspected human carcinogen [25]. Benzene is also a well-documented immunotoxic substance. Reported adverse effects on the immune system are decreases in lymphoid organ weights, antibody production, cell-mediated immunity, and host resistance to infections and to tumours [24]. Benzene is also a known leukemic agent in humans [26-28]. Finally, benzene and its metabolites inhibit both nuclear and mitochondrial replication and transcription in mice [27]. Moreover, during

metabolisation both benzene and its metabolites are converted to reactive species that covalently bind to macromolecules like DNA, RNA and proteins [27]. The potential danger of VOCs to marine organisms therefore lies mainly in chronic exposure to low levels, which may result in immunosuppression and carcinogenesis.

Once it has been established that a given chemical poses a threat, it remains to be determined what levels of contamination are acceptable in the marine environment. Van Leeuwen *et al.* [23] used Quantitative Structure Activity Relationships (QSARs), extrapolation of toxicity data and equilibrium partitioning for the assessment of the effects of narcotic industrial pollutants. The extrapolation of toxicity data generated by QSARs was used to derive safe levels for water, sediment and biota. Another, more pragmatic, approach is described by Mathiessen et *al.* [29] who applied a safety factor of 100 to acute toxicity data to establish safe levels of chronic exposure. Whereas the latter approach results in safety levels for the water column, the former model allows the calculation of internal toxic concentrations (ITCs) in fish tissues, which is useful for the interpretation of biomonitoring data. However, the usefulness of the model hinges on the applicability of the equilibrium-partitioning theory and its relation with octanol-water partitioning.

The present study aims at determining concentrations of a number of priority VOCs in organisms from the Southern North Sea and at studying their possible relation to geographical, temporal and/or biological parameters. In addition, the use of organisms to monitor these compounds in the marine environment will be discussed and the observed contamination levels will be compared with proposed safety levels.

3.1.2 Materials and Methods

Sampling

Samples were taken on board the Belgian oceanographic research vessel '*Belgica*' at six different locations (Fig. 3.1.1) using beam-trawling over a period of one year (five campaigns: April, May, June, October and December). Two sampling points (120 and 780) were situated near the coast (4-15 km), two (421 and 435) were situated further away from the coast (35-40 km), one sampling station (B07) was situated in the mouth of the Scheldt estuary and one (800), at 80 km from the coast, was selected as a reference point. Samples were processed as swiftly as possible to avoid contamination and losses.

Sampling was done in accordance with the guidelines of OSPARCOM [14]. Immediately after sampling, the undissected fish and shellfish were stored at -28 °C in closed containers and in the absence of organic solvents. Upon their arrival at the institute the samples were transported to an airtight freezer located in a solvent-free area.

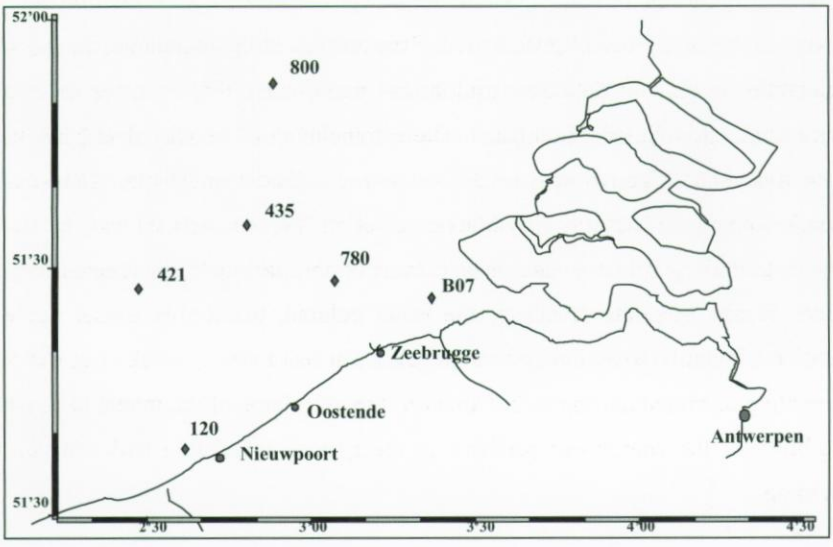

Figure 3.1.1: Sampling stations on the Belgian and Dutch continental shelfs.

Analytical methodology

A detailed description of the analytical methodology is given elsewhere [21]. Briefly, biological tissue is first homogenised (at 0° C) using an ultra-turrax blender and transferred to a 25-ml EPA vial. After addition of 15 ml of water and the internal standard (1,1,1-trifluorotoluene), the homogenate is treated in an ultrasonic bath (20 min at 0°C) to further disrupt the tissue. The glass vessel is then connected to a Tekmar (Tekmar, Cincinatti, USA) LSC 2000 purge and trap apparatus coupled to a gas chromatograph-mass spectrometer (GC-MS). The volatiles are forced out of the tissue by purging with a stream of helium gas while heating at 70°C and trapped onto a Vocarb 4000 sorbent trap. After purging, the trap is backflushed while being rapidly heated to 250 °C and the analytes are desorbed and, next, trapped in a cryofocusing module (-120°C) connected to

the analytical column (Restek, RTx-502.2, 60 m, 0.32 mm i.d., 1.8 µm film). The analytes are injected into the column by rapidly heating the module from -120°C to 200 °C in 0.75 min. Temperature programming of the GC and data acquisition were started simultaneously. The temperature of the GC oven was held at 40 °C for 2 min and then linearly increased from 40 °C to 200 °C at 10 °C/min. This temperature was then held for 5 min. Helium with an inlet pressure of 16 psi was used as the carrier gas.

The target compounds were identified on the basis of their retention times and mass spectra and quantified using the total mass of selected ions (Fig. 3.1.2). The ion trap detector was operated in the electron ionisation (EI) mode with the multiplier voltage set at 2400 V, the axial modulation (A/M) amplitude at 3.5 V and the emission current at 12 µA. The manifold temperature was set at 220 ° C. The mass range was 50-250 amu and the scan rate, 1000 ms. The filament delay was 180 s, and a mass defect of 50 mmass / 100 amu and a background mass of 55 amu were selected. Detection limits varied between 0.005 ng/g wet weight (1,2-dichoroethane, 1,1-dichloroethane and tetrachloromethane) and 0.2 ng/g wet weight (chloroform) depending on the background levels and the amount of sample [21].

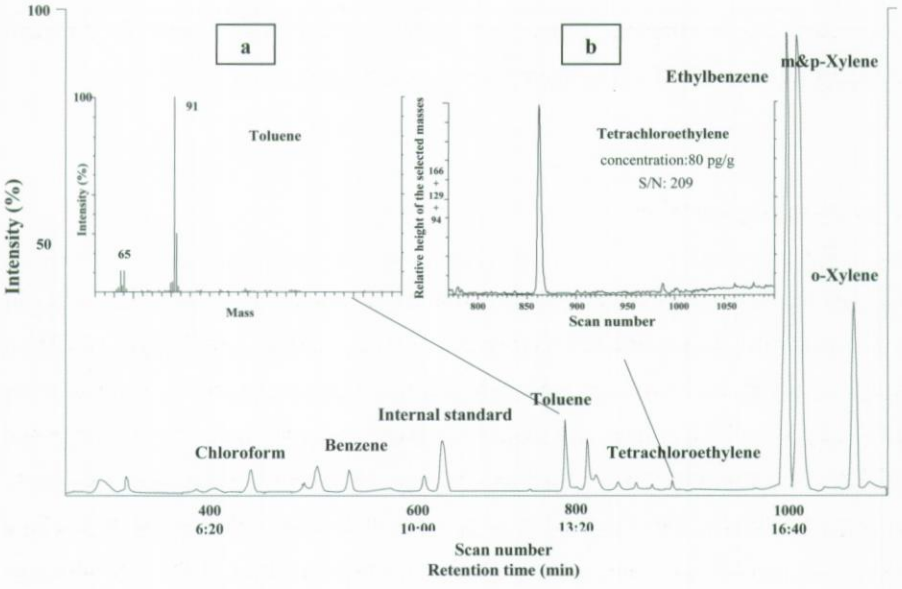

Figure 3.1.2: Representative total ion chromatogram of VOCs in a shrimp (*Crangon crangon*) sample with the mass spectrum of toluene (insert a) and the selected ion chromatogram of tetrachloroethylene (insert b).

Statistical analysis

For the statistical analysis, samples were separated according to species, tissue, sampling date and sampling location. Here, the present data set was combined with a previous one, which contained concentration data for dab and whiting from two sampling stations (120 and 800) [21]. For values below the detection limits, values equal to half these limits were used. A total of 237 statistical cases (a unique combination of concentrations, sampling time, location, species and tissue type) were considered for all 12 individual VOCs (statistical variables). Occasionally, in order to perform statistical tests that require a normal distribution, a logarithmic transformation of the original data set was used.

The Kolmogorov-Smirnov test (KS test) was used to determine whether a distribution was normal. The P value of the test was obtained with the Dallal and Wilkinson's approximation to Lilliefors' method [30]. In addition, normal probability plots (NPPs) were used to study the distribution of the data.

To distinguish specific groups of samples, a cluster analysis was performed. This was done by an average-linkage clustering (unweighted-pair group average) with between-group linkage based on squared Euclidian distances. To study underlying relationships between samples, a principal component analysis (PCA) was executed. Principal components were extracted when Eigenvalues were greater than one.

3.1.3 Results and discussion

Levels of VOCs

CHCs The concentrations of the CHCs, with the exception of chloroform were, in general, lower than those of the MAHs (Fig. 3.1.3). The 75 percentiles (75P) of all CHCs, with the exception of chloroform, were below 2 ng/g wet weight and the medians were below 1 ng/g for all the species and tissues that were analysed. Tetrachloromethane and 1,1-dichloroethane could not be detected (<0.005 ng/g wet weight) in a significant number of samples. For the other CHCs, except chloroform, the 75Ps varied between 0.02 ng/g for trichloroethylene and 1.5 ng/g for tetrachloroethylene with concentrations generally increasing in the order 1,1-dichloroethane < tetrachloromethane < trichloroethane < trichloroethylene < tetrachloroethylene < 1,2-dichloroethane. The 75Ps for chloroform, on the other hand, varied between 0.9 and 3.6 ng/g wet weight. Pearson

and Mc Connell [16], Dickson and Riley [17], Ferrario *et al.* [18] and Gotoh *et al.* [20] also found that the levels of chloroform were generally higher than those of the other CHCs. The only exceptions were eggs of marine birds from the Irish Sea, where the concentrations of the other CHCs were equal to or even higher than those of chloroform were [16]. The higher concentrations of chloroform in organisms are most likely related to higher concentrations in the water. This hypothesis is supported, for the Belgian continental shelf, by the findings of Dewulf *et al.* [31]. These authors indeed found higher water concentrations of chloroform and suggested that this could be the result of biogenic production of chloroform by marine algae. However, in contrast to the other CHCs, chloroform is also (inadvertently) formed during chlorination of drinking water, municipal sewage and cooling water [10]. Therefore, both its use in the chemical industry and the above inadvertent formation may well dominate the natural sources in an industrialised region, as is the case for the North Sea.

For the other CHCs, concentrations found in this study are similar to those reported in the literature (Table 3.1.1). There appear to be no large differences in the concentrations on a species or tissue type basis with two exceptions: the concentrations of tetrachloroethylene and 1,2-dichloroethane are significantly higher in the liver of dab than in muscle tissue. The cause of this dependence is not clear but is probably related to the intrinsic properties of the chemicals and the tissues concerned, and the way in which the organism was exposed. For instance, 1,2-dichloroethane showed a preference for liver and adipose tissue after oral administration but not after inhalation exposure [32]. Tetrachloroethylene, on the other hand, shows a tendency to accumulate in lipid-rich tissues such as the liver; this uptake is proportional to the exposure levels [33]. However, in general, the concentrations of CHCs are thought to be related to those in the water column through a process of physico-chemical partitioning and to be, therefore, directly related to the chemical properties of the compound of interest (see below).

MAHs For the MAHs, the 75Ps varied between 0.4 ng/g for benzene in shrimp and 28 ng/g for toluene in *Mactra stultorum*, and the median values varied between 0.08 ng/g wet weight for benzene and 22 ng/g for toluene for the same species, respectively (Fig. 3.1.3). That is, the concentrations were at least an order of magnitude higher than those of the CHCs. The concentrations of the C_2-substituted benzenes in Fig. 3.1.3 show closely

related patterns. This suggests that they are correlated, i.e. have a common source (see below).

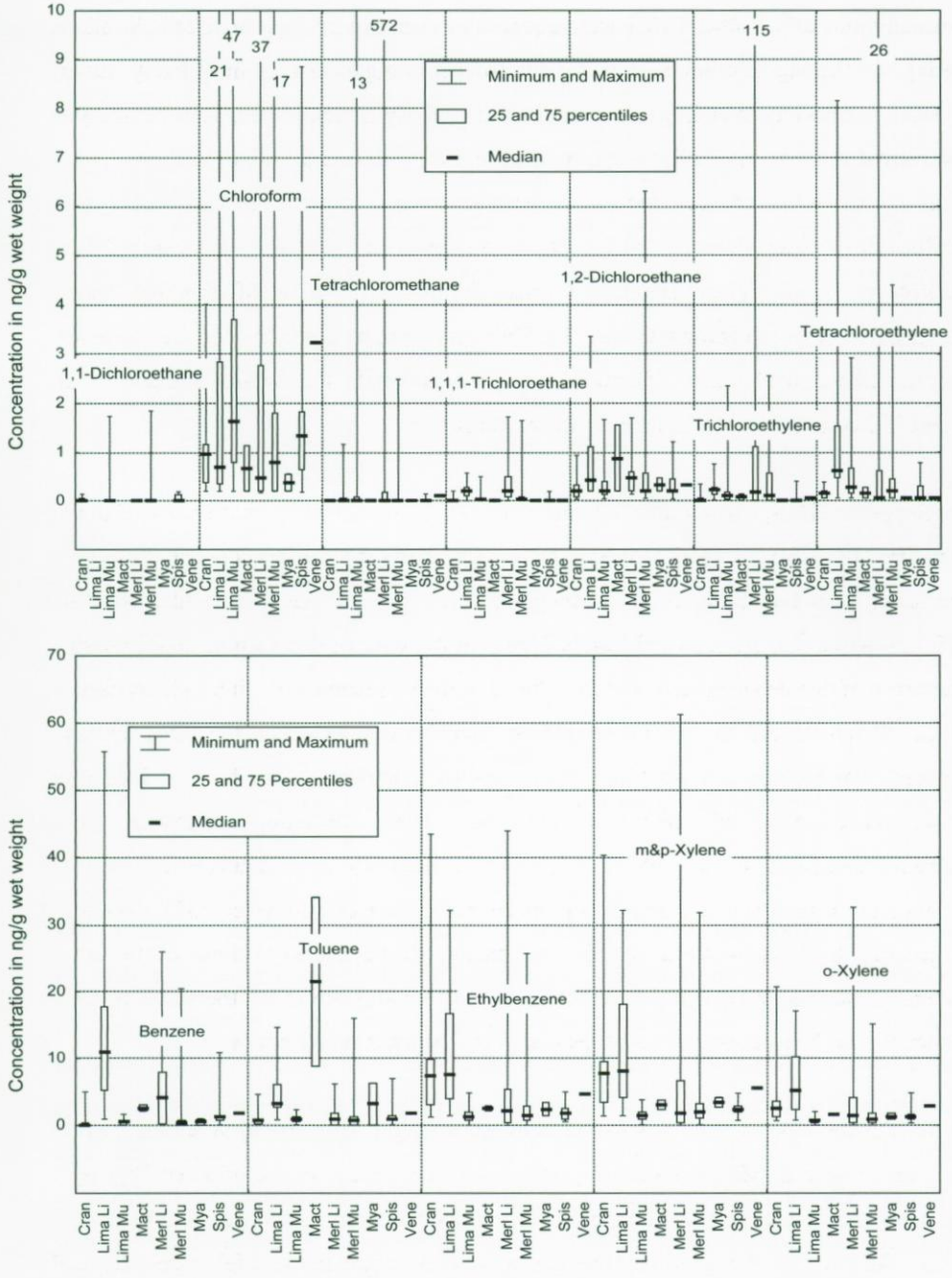

Figure 3.1.3: Concentrations in ng/g wet weight of CHCs (top) and MAHs (bottom) in *Crangon crangon* (Ccran), *Limanda limanda* liver (LimaLi), *Limanda limanda* muscle (Lima Mu), *Mactra stultorum* (Mact), *Merlangius merlangus* liver (MerlLi), *Merlangius merlangus* muscle (MerlMu), *Mya truncata* (Mya), *Spisula subtruncata* (Spis) and *Venerupis pullastra* (Vene).

No such similarity was found for benzene and toluene, which have concentrations that are sometimes higher, and sometimes lower than those of the C_2-substituted benzenes. The concentrations of MAHs in fish liver were consistently higher than in muscle tissue, especially for dab. These differences are probably related to metabolisation, because MAHs are known to be readily metabolised in organisms [34-37]. Furthermore, elimination of MAHs from organisms appears to be fairly rapid once exposure has ceased [34-37].

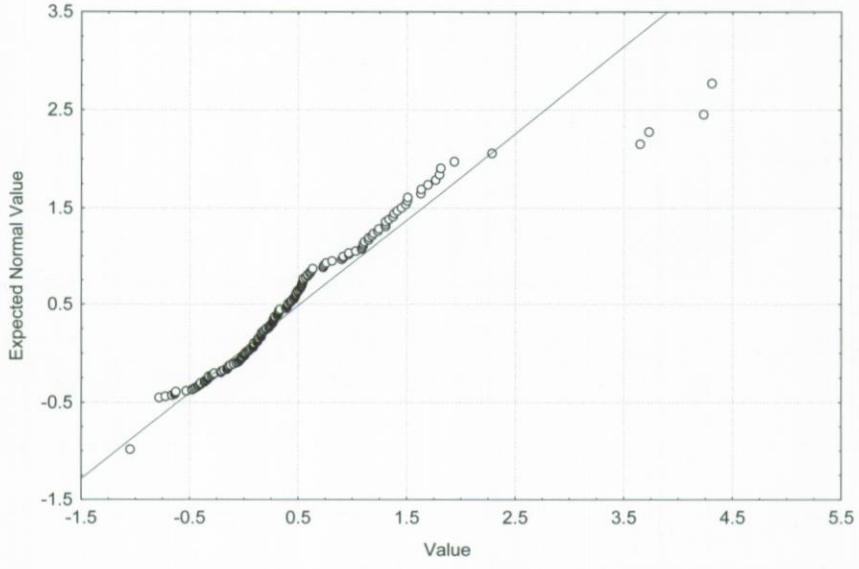

Figure 3.1.4: Normal probability plot for the trichloroethylene data of the present study.

As with the CHCs, the observed MAH concentrations in biota are related to the concentrations in the water column, as will be discussed below. Literature data on concentrations of MAHs in marine organisms are rather sparse. Ferrario *et al.* [18] reported concentrations of benzene, toluene and ethylbenzene in clams (*Rangia cuneata*) and oysters (*Crassostrea virginica*) from the estuary Lake Ponchartrain (USA). Benzene exhibited the highest concentrations in both clam (260 ng/g wet weight) and oyster (220 ng/g wet weight). The concentrations of toluene and ethylbenzene were significantly lower (maximum, 18 ng/g wet weight). No explanation was given for these differences, but the authors assumed that the contaminants were from anthropogenic origin. Since the concentrations in sediment were also higher for benzene, the higher concentrations in the

invertebrates were explained by a higher environmental load. Yasuhara and Morita [19] reported concentrations of benzene, ethylbenzene, o-xylene, p-xylene and m-xylene in *Mytilus edulis* (blue mussel) from two coastal locations in Japan. The concentrations ranged from 7.34 µg/g wet weight for benzene to 0.25 µg/g wet weight for ethylbenzene. The concentrations reported in the literature are high compared to those found in the present study. We observed at least 20-fold lower concentrations for benzene in the different species of marine clams and the concentrations of the other MAHs generally were about 10-fold lower.

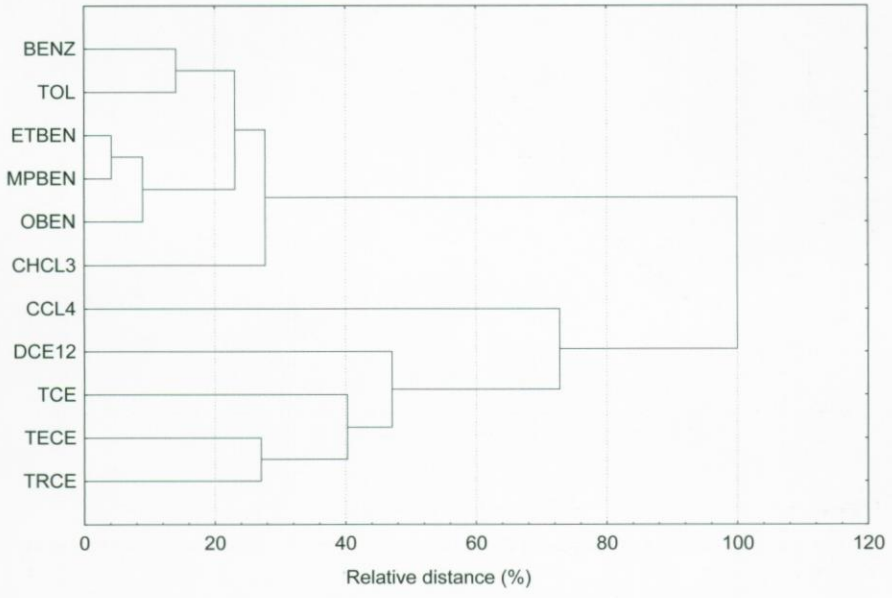

Figure 3.1.5: Horizontal hierarchical tree-plot representing the variable-wise cluster analysis of VOCs in marine organisms. (BENZ = benzene, TOL = toluene, EBEN = ethylbenzene, MPBEN = m&p-xylene, OBEN = o-xylene, CHCL3 = chloroform, CCL4 = tetrachloromethane, DCE12 = 1,2-dichloroethane, TCE = trichloroethylene, TECE = tetrachloroethylene and TRCE = trichloroethylene).

Statistical analysis

The data from the original set did not show a normal distribution, as was determined by the KS test and the NPPs. However, after logarithmic transformation and resubjection of the transformed data set to the KS test, the data sets for all MAHs and chloroform passed the test. Further evaluation of the distribution with NPPs showed that for trichloroethane, trichloroethylene and tetrachloroethylene, deviations from the normal distribution were

primarily caused by a few outliers (Fig. 3.1.4) and that the distributions for 1,2-dichloroethane and tetrachloromethane were mainly biased because of a large number of undetectable levels. However, since earlier observations showed that VOCs tend to be normally distributed for species from one batch [21], a normal distribution was generally assumed. The data for 1,1-dichloroethane were omitted from the data set as too few results were above the limits of detection.

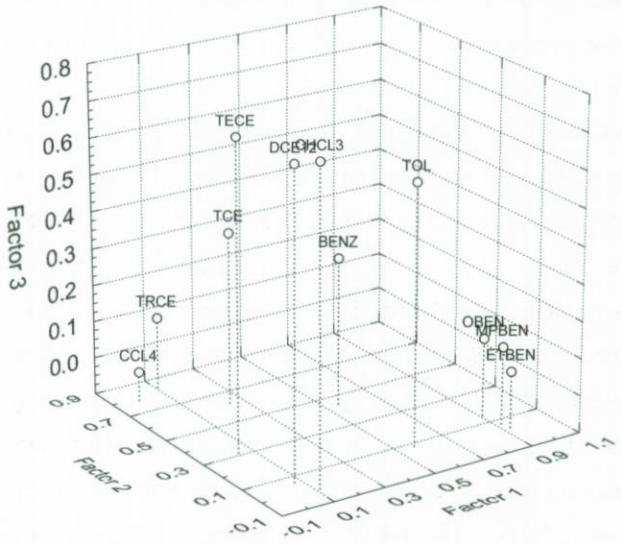

Figure 3.1.6: Three-dimensional plot of the factor loadings for the different VOCs after varimax rotation.

Cluster analysis was performed both case-wise and variable-wise. The variable-wise analysis resulted in two large clusters, one containing the MAHs and chloroform and the other, the rest of the CHCs (Fig. 3.1.5). The clustering is most probably the result of different concentrations, as is suggested by the higher levels of chloroform compared to the other CHCs. Nevertheless, within this cluster there is a clear distinction between chloroform and the MAHs. The latter clustered in two separate groups, benzene and toluene, and the C_2-benzenes. The distances for these groups were small which certainly suggests a common source. Furthermore, a correlation analysis of both clusters revealed that the concentrations of m&p-xylene and o-xylene correlated significantly with each other ($r = 0.87$) and with ethylbenzene ($r = 0.86$ and $r = 0.82$, respectively). The same was

true for benzene and toluene (r = 0.63). For the cluster representing the rest of the CHCs, only trichloroethylene and tetrachloroethylene appeared to cluster and even so not to the same extent as, for instance, the C_2-benzenes. For the rest, analysis of this group was hampered by a rather large number of undetectable levels; this was especially true for tetrachloromethane.

The case-wise analysis did not allow specific groups to be distinguished on the basis of species type, tissue type, sampling station or sampling date. A picture similar to the above was obtained with the PCA. Here, three factors were identified with Eigenvalues greater than 1. They contributed 40, 16 and 12%, respectively of the total variance of all samples. From the factor loading plot after varimax rotation (Fig. 3.1.6) it was clear that Factor 1 was mainly determined by the C_2-substituted benzenes, Factor 2 by tetrachloromethane, trichloroethylene and 1,1,1-trichloroethane, and Factor 3 mainly by chloroform, tetrachloroethylene and 1,2-dichloroethane. Benzene contributed to each factor to approximately the same extent, while toluene contributed mainly to Factors 3 and 1. This means that all individual VOCs, except toluene and benzene, were closely related to only one factor. Furthermore, the largest variability in the database (40%) can be attributed to differences in concentration of, especially, ethylbenzene and the xylenes and, to a lesser extent, benzene and toluene. This first principal component further allows a distinction to be made between MAHs and CHCs, since the latter hardly contribute to this factor.

When the factor scores of all samples are considered (Fig. 3.1.7), no distinct clusters of samples could be distinguished on the basis of species type, tissue types, sampling location or sampling time. As for the cluster analysis, the only explanation of the observed differences was concentration differences of the three groups identified above. For instance, the encircled cluster in Figure 3.1.7 (cases 112-115) with a high score for Factor 2 is characterised by high concentrations of tetrachloromethane, trichloroethylene and, to a lesser extent, 1,1,1-trichloroethane. These are liver tissue samples of whiting from the same location and the same date. Although they cluster because of the high concentrations mentioned above, they do not cluster with other samples that have the same characteristics (species type, tissue type, sampling location and sampling time).

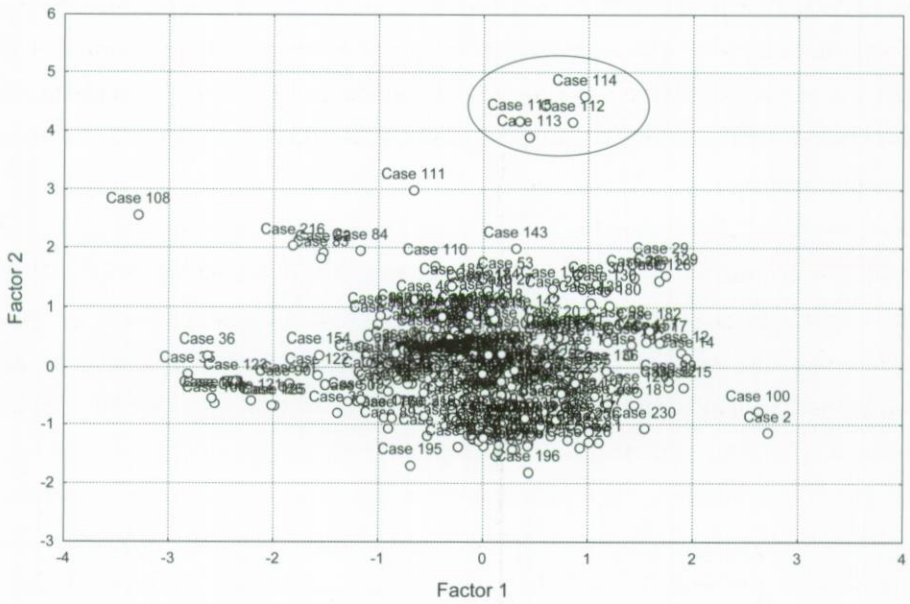

Figure 3.1.7: Factor scores for all samples (Factor 1 vs. Factor 2), with a distinct cluster of samples encircled.

At the outset of this study, we assumed that a number of causes could result in differences between the samples. Among these were distance to the coast (influence of land-based emissions), the vicinity of point sources (such as the Scheldt estuary), seasonal variations (such as the increased used of fossil fuels in winter) and biological parameters (such as preferential accumulation in certain tissues, metabolisation, food web effects). Somewhat surprisingly, despite the large number of data, neither the cluster analysis nor the PCA allowed the samples to be distinguished. However, the correlation analysis and both ordination analyses show that the concentrations of C_2-substituted benzenes are closely related to each other. The largest emission source of ethylbenzene and the xylenes is gasoline [10,34,37,38] and the correlation observed for these chemicals can possibly be related to this common source. This would also mean that the principal source of ethylbenzene and xylenes in marine organisms is the use of fossil fuel. The latter is also a known source of benzene and toluene and more than likely explains the grouping observed in the cluster analysis and the PCA. Another possible source is suggested by Dewulf *et al.* [31]. The authors observed higher levels of MAHs, compared to the CHCs, in water and air samples from the same region and attributed this to anthropogenic emissions from oil transport in this coastal area. In addition, the fossil fuel source is also

one of the main differences between the MAHs and the CHCs. Chloroform is, in this context, an exceptional compound as it is inadvertently formed during chlorination of water (see earlier) and has known natural sources. Chlorination of water is potentially the largest source of chloroform for the environment [10]. Finally, the lack of differences between the various sampling stations allows to suggest that, for all practical purposes, the part of the Belgian and Dutch continental shelf considered in this study can be regarded as one zone, i.e. an area that is influenced by the same sources, as far as VOC concentrations are concerned. The absence of seasonal differences suggests that the area, and therefore the organisms, is subjected to the same sources the year round and that the sources are essentially constant in nature.

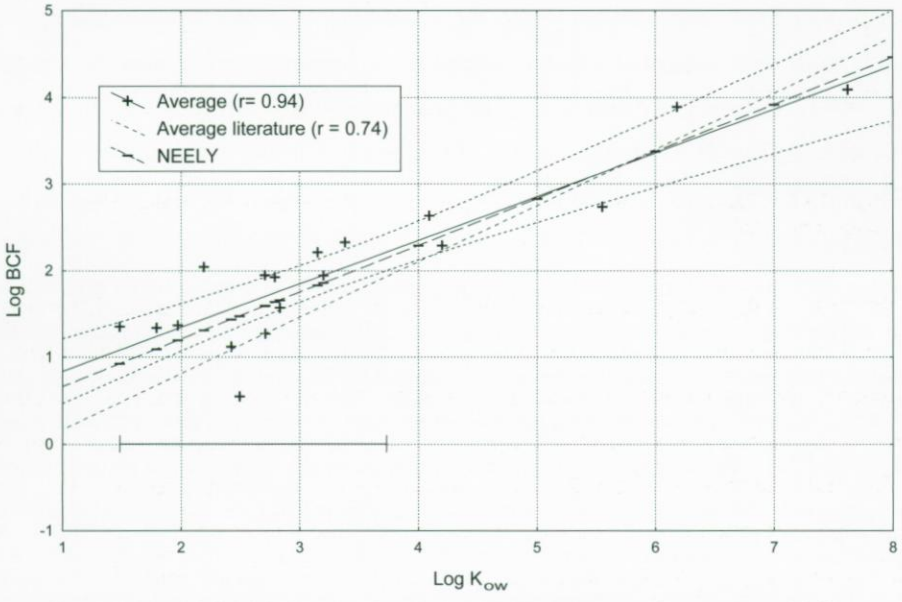

Figure 3.1.8: Relationship between the calculated BCFs and K_{ow}, and comparison with literature data. The data from the present study were within the range indicated by the barred line.

Bioconcentration and hazard assessment

In order to evaluate the possible consequences of the VOC concentrations found in marine organisms, described in the previous sections, one can use the hazard assessment proposed by Van Leeuwen *et al.* [23]. However, as was mentioned earlier, the model hinges on the applicability of the Equilibrium Partitioning Theory (EPT). According to the EPT, concentrations of chemicals, such as VOCs, in organisms originate from those in the water column through a process of physico-chemical partitioning. That is, the EPT

assumes passive partitioning of a chemical compound between the aqueous phase and a lipid or a lipid-like organic phase [23]. The resulting partition coefficient, which is equal to the ratio of the concentrations in the organism (C_{org}) and the water (C_w), is called the Bioconcentration Factor (BCF):

$$BCF = C_{org} / C_w \quad [1].$$

This partition coefficient is supposed to be an intrinsic property of the compound and can, as a result, be related to its octanol-water partition coefficient, K_{ow}. Neely et al. [39] and, subsequently, several other authors [40-42] demonstrated that BCF and K_{ow} are linearly related according to:

$$Log\ BCF = a\ log\ K_{ow} + b \quad [2].$$

with *a* the regression coefficient and *b* the y intercept. The data obtained during the present study were compared with the average water concentrations reported by Dewulf *et al.* [31] for the southern North Sea and BCFs were calculated for all VOCs. Plotting the logarithm of these BCFs against log K_{ow} indeed resulted in the linear relationship ($r = 0.42$) predicted by Eq. 2. This becomes especially evident when the data for higher K_{ow} values reported by Neely *et al.* [39] are included in the picture (Fig. 3.1.8). The larger number of data points, spread over a larger K_{ow} range results in a much better correlation ($r = 0.94$). Moreover, the observed slope is essentially the same as the one reported by these authors. However, plotting the average of the BCFs reported in the literature [10] resulted in a slope that is lower than those obtained with our data and those of Neely *et al.* [39] (Fig. 3.1.8). This suggests that the BCFs reported in the literature are somewhat too low, especially for the VOCs with a log K_{ow} of less than 2.8. One explanation could be the use of nominal instead of actual concentrations. BCFs reported in the literature are often the result of laboratory experiments in open systems and nominal concentrations can easily be too high due to the high volatility of the compounds of interest [37]. Even so, the observed relationship indicates that VOC concentrations in the water column are indeed reflected in the organisms and suggests that the EPT can be applied.

On the basis of the above observations one may conclude that the hazard assessment of Van Leeuwen *et al.* [23] can be used. These authors used QSARs, the extrapolation of toxicity data and equilibrium partitioning to assess the effects of narcotic industrial pollutants such as the target compounds of this study. The extrapolation of toxicity data

generated by QSARs was used to derive safe levels for water. The QSARs in their study were expressed as:

$$log\ NOEC = a'\ log\ K_{ow} + b'\ [3]$$

where NOEC is the no-observed-effect concentration, a' the regression coefficient and b' the y intercept. These concentrations were derived from literature data or, if no chronic toxicity data were available, estimated from acute toxicity data using acute/chronic ratios. The safety level was arbitrarily set at 95%. This implies that a threshold concentration is calculated which is unlikely to cause harm to 95% of the aquatic community. This calculated concentration, HC5, is the hazardous concentration that will affect, at most, 5% of the species. The $HC5_w$ for the water column was calculated from:

$$HC5_w = C_w\ x\ (1+1.85x10^{-6}\ K_{ow}) \qquad [4]$$

where $HC5_w$ is the total concentration in the water phase (including suspended matter) and C_w the concentration in the water column for a given K_{ow} that is unlikely to harm 95% of the population, calculated on the basis of the QSARs as given in Eq.3. The proportionality constant, a', relates to the average suspended matter concentrations in the area and their organic carbon content [23]. The internal tissue concentration, ITC or $HC5_{org}$, for the organisms was calculated from

$$HC5_{org} = 0.05\ x\ HC5_w\ x\ K_{ow} \quad [5]$$

where a lipid content of about 5% wt. in the organism is assumed.

Table 3.1.2: Comparison of the average tissue concentrations in pg/g of the present data set and the proposed safety level (HC5) with Cran (*Crangon crangon*), Mactra (*Mactra stultorum*), Mya (*Mya truncata*), Spis (*Spisula species*), Lim Li (*Limanda limanda* liver), Lim Mu (*Limanda limanda* muscle tissue), Mer li (*Merlangius merlangus* liver) and Mer Mu (*Merlangius merlangus* liver).

Compound	Cran	Mactra	Mya	Spis	Lim Li	Lim Mu	Mer Li	Mer Mu	HC5
MAHs									
Benzene	700	2500	550	2000	14000	500	5800	800	$5.2*10^6$
Toluene	900	21000	3200	1600	4800	950	1500	1000	$5.9*10^6$
Ethylbenzene	9800	2500	2400	2200	11000	1500	5200	2600	na
m&p-Xylene	9700	3000	3500	2500	11000	1500	6300	3200	$6.4*10^6$
o-Xylene	4100	1600	1300	1600	6000	700	3600	1500	$6.5*10^6$
CHCs									
1,1-Dichloroethane	40	nd	Nd	60	nd	140	5	100	$6.7*10^6$
Chloroform	1100	700	400	2600	3200	5400	2800	2000	$8.1*10^6$
Tertrachloromethane	8	5	5	20	200	450	43000	70	$9.8*10^6$
1,2-Dichloroethane	300	900	300	400	900	300	550	500	$6.7*10^6$
1,1,1-Trichloroethane	40	20	6	30	200	50	400	100	$8.8*10^6$
Trichloroethylene	70	80	20	60	200	200	13000	400	$8.7*10^6$
Tetrachloroethylene	200	200	60	200	1200	500	1300	350	$9.7*10^6$

nd = not detected, na = not available

Table 3.1.2 compares the calculated $HC5_{org}$ values and the average concentrations in the different organisms and tissues. The results show that, in no case, is the $HC5_{org}$ for the MAHs and CHCs exceeded. Most probably, this would have been true also for ethylbenzene if an $HC5_{org}$ had been available. Moreover, the observed averages are several orders of magnitude lower than the $HC5_{org}$. However, the present results may still cause concern because the hazard assessment does not take into account synergistic and, thus, more damaging effects. Despite the often high results, no definite statements can, as yet, be made concerning long-term effects such as carcinogenicity or immunosuppression. The number of data is too limited and the calculation of the HC5 is one approach amongst several and needs to be further evaluated. What is clear, however, is that additional research, especially with regard to the long-term consequences of small doses of VOCs is urgently required.

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3.2 Measurement of volatile organic compounds in sediments of the Scheldt estuary and the southern North Sea[††]

Summary

The concentrations and distribution of 13 priority volatile organic compounds (VOCs) were determined in sediments of the Scheldt estuary and the Belgian continental shelf, using a modified Tekmar LSC 2000 purge-and-trap system coupled to GC-MS. The method allows a sample intake of up to 50 g wet weight and detection limits are between 0.003 ng/g (tetrachloromethane) and 0.16 ng/g (m- and p-xylene). The repeatability (n=5) varied between 4% (benzene) and 17% (toluene) and the recoveries ranged from 59% (1,1-dichloroethane) to 99% (tetrachloromethane). Because of the nature of the contaminants, special attention was paid to analyte losses and contamination of the samples during storage aboard the research vessel. Spiked sediment samples were prepared in the laboratory and stored aboard under the same conditions as the environmental samples. The recoveries for these samples varied between 94% and 130%, which suggests that storage had no adverse effect on the samples. No detectable VOC concentrations were found for most of the sampling stations. However, in the Antwerp harbour area, significant concentrations of VOCs were found. The sorption behaviour as predicted from laboratory equilibrium partitioning experiments gives an indication of the *in situ* partitioning behaviour of VOCs. Although VOCs in sediments should, in general, not be regarded as a major problem in the marine environment, high local concentrations may be a cause of concern.

[††] From *Water Res.*, 35 (2001) 1478-1488.

3.2.1 Introduction

The presence and distribution of volatile organic compounds (VOCs) in marine and estuarine systems have so far received relatively little attention from the scientific community, as was recognised by the International Conferences on the Protection of the North Sea [1,2]. VOCs enter the marine environment through their use as solvents and in production processes; their formation during chlorination of drinking water and exploitation and use of fossil fuels [3,4]. Recent work showed the presence of VOCs in marine organisms from the Belgian coastal region at concentrations comparable to those of well-known contaminants such as PCBs [5]. The significance of these findings for e.g. reproductive success and survival of organisms is at present unknown. It can be assumed that organisms are mainly exposed to contaminants through contaminated water and by the ingestion of contaminated particles or sediment and through food. Dewulf et al. [6] determined 13 priority VOCs in water from the Belgian coastal region and found average concentrations ranging from 2.2 to 73 ppt over a period of 1.5 year. The water column can therefore be regarded as a potential source of VOCs for organisms, especially when one considers that these VOC concentrations are approximately 1000-fold higher than those of, for instance, individual PCB congeners [7]. The contribution of sediments to the presence of VOCs in organisms is at present unknown. Parameters reflecting the equilibrium partitioning over the different compartments (air, water, sediment and organisms), environmental degradation rates and intercompartment exchange velocity models provide information to establish models which can predict the environmental fate of organic pollutants [8]. A low sorption can be expected because of the low Kow (octanol-water partitioning coefficient) values of most VOCs and the low organic carbon content of marine sediments, since VOCs are thought to be mainly associated with the organic fraction of sediments [9]. Recent experiments seem to confirm this and sediments are, therefore, generally not regarded as major sinks for VOCs [3,9]. However, reported concentrations in sediments tend to be higher than those of the overlying water column [10,11]. Moreover, the nature of organic matter in soils, which are comparable to sediments, can vary substantially. The degree of aromaticity [12] or the polar-to-nonpolar group ratio (O+N)/C [13] influence the sorption equilibrium. Because of this and the inherent stability of VOCs, sediments cannot be ruled out as a local source of VOCs for organisms.

There appears to be no universally recognised and approved method for the determination of VOCs in sediment. A variety of methods were reported in the literature which are based on techniques such as solvent extraction [14,15], vacuum distillation [16,17] and supercritical fluid extraction (SFE) [21], but the most commonly used methods are based on either static [18-20] or dynamic [10,11,22-25] headspace techniques. The latter is sometimes preceded by a solvent extraction step, in which case the extract itself is then analysed using a dynamic headspace or purge-and-trap (P&T) technique [26,27]. Static headspace offers the advantage of lower cost, easy automation and rapid sample throughput [18]. The main disadvantage are the relatively high detection limits (LODs), although Bianchi and Varney [20] reported LODs below 0.5 µg/kg dry weight for VOCs in sediments. The best LODs are generally found for methods that use P&T techniques. For example, Al-Rekabi et al. [11] reported LODs of 40-50 ng/kg wet weight for various VOCs and Bianchi et al. [10] found LODs of 20-300 ng/kg dry weight when using a similar approach. The latter group determined a large number of VOCs in the Solent estuary (UK). The analyte recoveries and repeatabilities of the different methods vary considerably and depend on the methodology. Using solvent extraction or SFE, recoveries are generally better than 80% [14,21] with RSDs (Relative Standard Deviations) below 10%, while for vacuum extraction recoveries varied between 50 and 100% [17] and the RSDs between 20 and 30%. Voice and Kolb [19] obtained recoveries of over 70% and RSDs of 5-10% when using static headspace. These authors also demonstrated the superior performance of the latter technique compared to P&T. The large variability in both recoveries (30-100%) and RSDs (1 - >30%) of the P&T techniques is well known [10,11,20,23-25]. Operational parameters such as, especially, the purge temperature, influence the overall performance. Bianchi et al. [10] showed an improved performance of their method when the samples were purged at a temperature of 60 °C rather than 30 °C. The efficiency of the P&T technique appears not to be influenced by the sample composition or sample size [24], which is a distinct advantage.

For this work, a P&T method developed for the determination of 13 priority VOCs in biota [28] was evaluated for the analysis of sediment samples. This study is a follow up on recent studies on the analysis, concentration and distribution of VOCs in water, air and organisms in the Belgian coastal area that were initiated in the framework of a national research programme. It was undertaken in order to study the importance of sediment as a source for VOCs in organisms, to test the applicability of the methodology and to

establish concentrations in sediments of the Scheldt Estuary and the Belgian continental shelf. In addition the partitioning behaviour of VOCs in sediment was further investigated.

3.2.2 Materials and methods

Materials

All materials used for the various experiments and analyses were of research-grade quality. The chlorinated hydrocarbons (CHCs), chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene, and the monocyclic aromatic hydrocarbons (MAHs) benzene, toluene, ethylbenzene and the xylenes, were all from Merck (Darmstadt, Germany). Selection of the VOCs was based on international priority pollutant lists [1,2]. They were used without further purification. Methanol (Instra-analysed, J.T. Baker, Phillipsburg, USA) was used to prepare standard solutions. 1,1,1-Trifluorotoluene (Aldrich, Milwaukee, USA) was used as internal standard (IS). Vocarb 4000 traps (8.5 cm Carbopak C, 10 cm Carbopak B, 6 cm Carboxen 1000 and 1 cm Carboxen 1001) were obtained from Supelco (Bellefonte, USA) and used as adsorption traps (1/8" OD). Water used for the preparation of blanks and standards was obtained from J.T. Baker. Extra-pure sea sand for blanks and calibration was obtained from Merck.

Apparatus

A microprocessor-controlled P&T system, the Tekmar LSC-2000 (Tekmar, Cincinati, USA), was coupled to a GC-MS (Finnigan Magnum Ion Trap MS, Finnigan, San José, USA) via a heated transfer line terminating in a cryogenic focuser at the GC end. The internal lines of the P&T were constructed from glass-lined stainless steel and the transfer line and internal lines were connected via a heated 6-port switch valve. The standard needle sparger of the Tekmar was replaced with a system consisting of two needles (purge gas inlet and outlet) and a moisture trap, which was a 40-ml vial cooled to –10 °C (Roose and Brinkman, 1998b). The 40-ml open-hole screw cap vials (moisture trap and sample vials) and PTFE/silicone liners were from Alltech (Deerfield, USA).

Sampling and storage

Samples were collected during two periods (March 1997 and March 1998) aboard the Belgian oceanographic research vessel 'Belgica' at different locations (Figure 3.2.1)

using a Van Veen grab-sampler. Sampling locations were selected from among those of an ongoing sampling programme in such a way that both a more remote and a more coastal location were represented and that the salinity gradient in the Scheldt estuary was covered. Immediately after sampling, the sediment samples were taken from the central portion of the grab using all-glass vials, and without a headspace. The vials were immediately closed with a PTFE-lined screw cap (Alltech). Samples were stored at 4 °C in the absence of organic solvents. Upon their arrival in the laboratory the samples were stored in an airtight refrigerator located in a solvent-free area in a separate building.

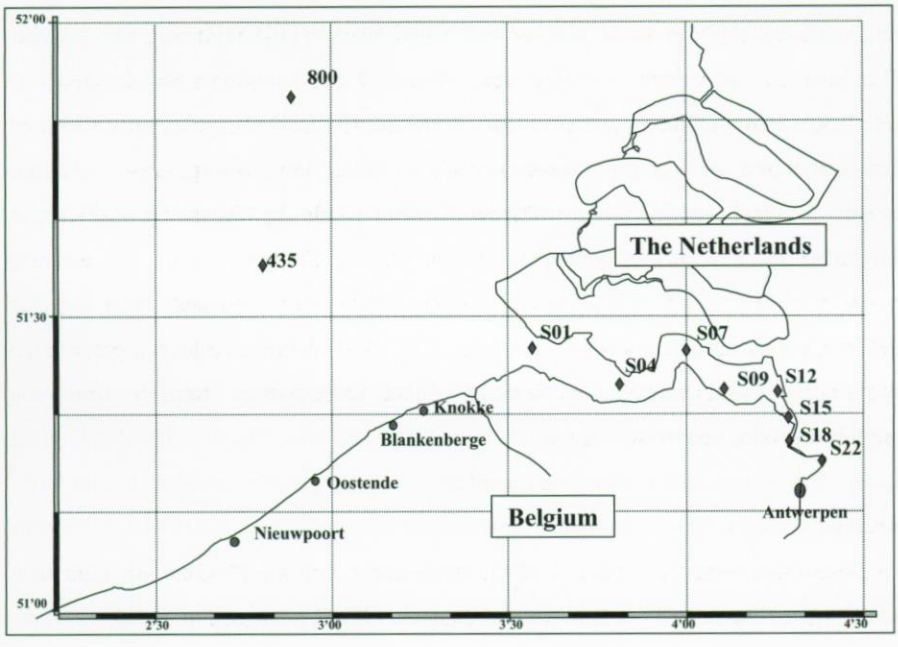

Figure 3.2.1: Sampling locations on the Belgian continental shelf and in the Scheldt estuary.

Analytical procedure

Preparation of blanks Water specially prepared for the analysis of VOCs (J.T. Baker) and extra-pure sea sand (Merck) were used to prepare blanks and standard solutions (see below). Both water and sediment were pre-treated by heating to 90°C with simultaneous purging with helium (N 7.0, l'Air Liquide, Liège, Belgium) or nitrogen (N 6.0, l'Air Liquide) in a glass sparger. As a routine, the latter were continuously purged during storage with helium or nitrogen. For the preparation of blank samples, 15 ml of the treated

water were drawn up in a 100-ml syringe and 1 µl of the internal standard was added by inserting a 10-µl HPLC syringe in the opening of the 100-ml syringe. The water was added to approx. 30 g of the blank sediment and the entire sample was then taken through the complete analytical procedure.

Preparation of standard solutions and spiked sediment samples A detailed description of the preparation of standard solutions is given elsewhere [5]. For calibration of the procedure, 1 µl of a methanolic solution containing 0.4-0.8 ng/µl of the various target compounds was injected with a 10-µl syringe in a 100-ml syringe containing 15 ml of blank water (see above). Next, 1 µl of a methanolic solution containing the internal standard (about 0.4 ng/µl) was also introduced into the 100-ml syringe with another 10-µl syringe. The water was then injected into a 40-ml sample vial filled with approx. 30 g of blank sediment and, after an equilibration period of 1 hour, the sample vial was connected to the on-line P&T set-up, pre-concentrated and analysed by GC-MS. An identical procedure was used for spiked sediment.

Sample pre-treatment and analysis

Approx. 30 g of sediment were transferred to a 40-ml sample vial. After the addition of 15 ml of organic-free water and internal standard, the vial was closed with a PTFE-lined screwcap. The glass vessel was then coupled to an impinger connected to the P&T system. The volatiles were forced out of the sediment by purging the sample for 30 min with a 20 ml/min stream of helium at 70°C (water bath). The analytes were trapped on a Vocarb 4000 sorbent trap mounted in the P&T apparatus, at a temperature of 45 °C. After purging, the trap was backflushed while being rapidly heated to 250 °C and the analytes were desorbed into a cryofocusing module cooled to -120°C and connected to the GC column. The analytes were injected into the column by rapidly heating the cryofocusing module from -120°C to 200 °C in 0.75 min. Separation was done on a 60 m x 0.32 mm i.d. (1.8 µm film) Restek, RTx-502.2 column. Temperature programming of the GC and data acquisition were started simultaneously. The temperature of the GC oven was held for 2 min at 40 °C and then increased from 40 °C to 200 °C at 10 °C/min. The final temperature was held for 5 min. Helium was used as the carrier gas with an inlet pressure of 16 psi.

Table 3.2.1: Retention windows, selected masses, recovery, repeatability and LOD of the target compounds.

Compound	Retention window (min)	Selected masses	Recovery[1] (%) (n=5)	RSD (%) (n=5)	LOD[2] (pg/g wwt)
1,1-Dichloroethane	4:30-4:50	63, 64	59	23	4
Chloroform	6:10-6:30	83, 85	88	16	90
Trichloroethane	6:40-6:60	61, 97, 99	97	17	4
Tetrachloromethane	7:00-7:20	117, 119	99	17	3
1,2-Dichloroethane	7:10-7:30	62	97	17	20
Benzene	7:10-7:30	78	92	4.3	50
Trichloroethene	8:00-8:20	60, 130	95	16	30
Toluene	9:45-9:65	91	86	17	100
Tetrachloroethene	10:40-10:60	91, 105	92	15	50
Ethylbenzene	12:00-12:20	91, 106	82	14	60
m&p-Xylene	12:05-12:25	91, 106	82	14	200
o-Xylene	12:45-12:65	91, 106	81	14	50

[1] Recoveries for a sediment sample spiked with concentrations ranging from 280 to 580 pg/g depending on the compound.
[2] LOD calculate for an average sample intake of 30 g.

The target compounds were identified on the basis of their retention times and mass spectra and quantified using the total mass abundance of selected ions (Table 3.2.1). The ion trap detector was operated in the electron ionisation (EI) mode with the multiplier voltage set at 2550 V, the axial modulation (A/M) amplitude at 4.0 V and the emission current at 13 μA. The manifold temperature was 220 ° C. The mass range was 50-250 amu and the scan rate 1000 ms. The filament delay was 180 s, and a mass defect of 50 mmass / 100 amu and a background mass of 45 amu were selected.

Analytical quality assurance

A blank sample was run with each series of samples. The peak heights of the analytes in the blank were compared with those in the standard solution used for calibration. Peak heights in the blank should at least be ten times lower than those in the standard solution (warning limit) and never be less than five times lower (control limit). A second quality assurance measure (QA) was to monitor the response factors of the different VOCs during the analysis of the standard solution used for calibration. Deviations of over 30% (± 2 sd) from the median response factor were considered as out of control. When the results of a test were out of control, a standard solution was treated as a sample and analysed as an

internal reference material (IRM). The test provides a way to determine whether the problem is MS or P&T related.

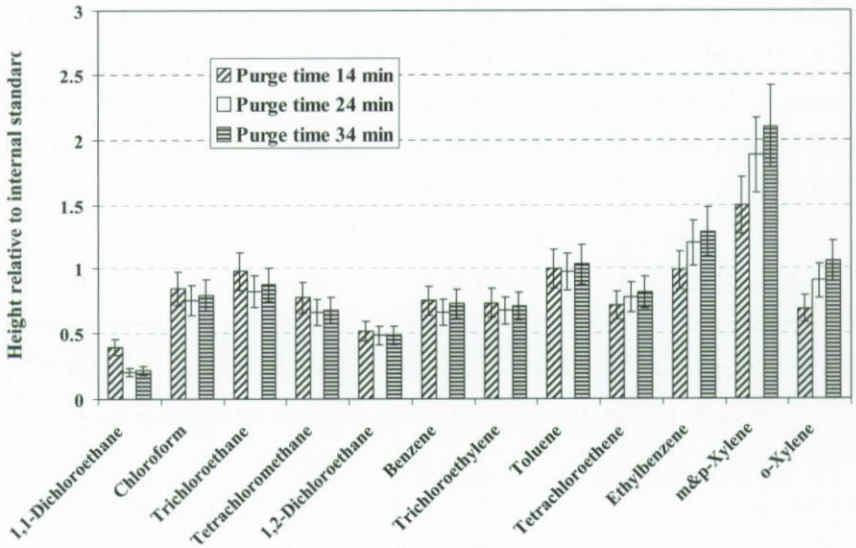

Figure 3.2.2: Effect of purge time on recovery of the analytes given in order of elution (n=3). The error bars represent the standard deviation.

3.2.3 Results

Analytical data

The P&T method developed earlier for the determination of 13 priority VOCs in biota [28] was slightly modified, viz. with respect to the sample preparation and sample intake. As there were practically no changes in the operational parameters of the earlier set-up, only the purge time was evaluated. To this end a spiked sediment sample was purged under otherwise identical conditions for different periods of time. The results are presented in Figure 3.2.2. For the compounds up to toluene no significant differences were observed between purge times of 14, 24 and 34 min, with the exception of 1,1-dichloroethane. The latter is clearly affected by longer purge times, presumably because 1,1-dichloroethane, with its high volatility, will break through the sorbent under these conditions. For the less volatile compounds such as tetrachloroethene, ethylbenzene and the xylenes, a substantial difference was observed between a purge time of 14 min and purge times of 24 min and 34 min. A purge time of 34 min was selected for all further work. To further test the method, both the repeatability (short-term precision) and the

recovery were determined by analysing five replicates of a spiked sediment sample. The results of the tests are given in Table 3.2.1. The recoveries varied between 80 and 99%, with one exception, 59%, for the highly volatile 1,1-dichloroethane, which can be explained by the 34-min purge time (cf. above). The repeatability, calculated as the RSD of five independent analyses, was between 14 and 17% for all but two VOCs. The deviating result for benzene cannot easily be explained. The much higher RSD for 1,1-dichloroethane is due to its less efficient recovery caused by the prolonged purging. The LODs were calculated for a standard sample of 30 g and were based on the analytical blank (blank + 3 sd) or a signal-to-noise ratio of 3 [5]. Considering that blank values range from 0.02 ng/g for tetrachloroethene to 0.08 ng/g wet weight for m&p-xylene, the method allows the detection of individual VOCs in sediments at concentrations varying from 0.004 ng/g for trichloroethane, for which no background levels were found, to 0.2 ng/g wet weight for m&p-xylene, with the above mentioned background levels.

Sample storage

Because of the volatility of the compounds of interest, analyte losses and sample contamination during storage aboard the research vessel and in the laboratory may well occur. Both sampling and storage were devised in such a way that contamination and losses would be minimised. To obtain an idea of both hazards, spiked sediment samples and blank sediment samples were prepared in the laboratory and stored aboard under the same conditions as the grab samples (1997 campaign). The prepared samples were then transported to and stored in the laboratory together with the sediment samples, again under identical conditions. The analyte recoveries for the spiked sediment samples varied between 94% and 130% (median: 102%); that is, they were within two standard deviations of the expected 100% recovery which proved that storage had no adverse effect on the samples. This was further confirmed by the fact that no significant differences were found between a laboratory blank and a set of blanks that were stored together with the environmental samples aboard and in the laboratory.

Sediment samples

P&T-GC-MS was used to analyse the samples collected at ten stations on the Belgian continental shelf and in the Scheldt estuary during two surveys in 1997 and 1998. The results of the analyses for both surveys are given in Table 3.2.2. For most of the sampling stations no detectable concentrations of the target compounds were found in 1997. Only

at sampling station S22, in the industrial region of Antwerp harbour (Figure 3.2.1), a marked presence of VOCs could be demonstrated. Even so, only the less volatile members of the group were present in the sediment but there was no clear relation between vapour pressure and sediment concentration. As every sampling station was independently sampled twice and the two samples were analysed individually, an idea of the sampling variability could be obtained. For obvious reasons this was only done for station S22.

Table 3.2.2: Concentration range and medians between brackets (where applicable) for VOCs in pg/g wet weight for the different sampling stations in the period 1997-1998.

Compound	Sampling station									
	800	435	S01	S04	S07	S09	S12	S15	S18	S22
1,1-Dichloroethane	-	-	-	-	-	-	-	-	10[1]	-
Chloroform	-	100[1]	-	-	-	-	-	-	-	-
Trichloroethane	-	-	-	-	-	-	-	< 4 – 170 (90)	< 4 – 60 (15)	-
Tetrachloromethane	-	-	-	-	6[1]	4[1]	-	-	50[1]	-
1,2-Dichloroethane	-	-	-	-	-	-	-	-	-	-
Benzene	-	-	-	-	70[1]	70[1]	< 50 – 110 (90)	< 50 – 120 (100)	90 – 340 (200)	-
Trichloroethene	-	-	-	-	-	-	-	< 30 – 80 (40)	< 30 – 70 (50)	< 30 – 90 (60)
Toluene	-	-	-	-	-	-	-	< 100 - 750 (400)	280 – 810 (690)	180 – 910 (700)
Tetrachloroethene	109[1]	-	< 50 – 350 (240)	-	-	-	-	-	-	< 50 – 110 (80)
Ethylbenzene	-	-	-	-	-	-	-	70[1]	100[1]	< 60 – 130 (80)
m&p-Xylene	-	-	-	-	-	-	-	-	-	80 – 230 (150)
o-Xylene	-	-	-	-	-	-	-	30 – 50[2] (40)	-	< 50 –110 (90)

- Below detection limits as given in Table 3.2.1
[1] Cases where only one sample had levels above the LOD
[2] Lower LOD due to lower background levels or higher sample intake

Considering that the average repeatability of the analytical method is 15%, the variability as a result of sampling was negligible for compounds such as toluene (17%), with concentrations well above the detection limit. When concentrations are near the detection limits the variability markedly increased (30-80%). During the 1998 survey, the VOCs were detected at the sampling stations S12, S15, S18 and S22 upstream the Scheldt river,

with the highest concentrations being found at stations S15 and S18. During this survey every station was sampled five times and each sample was analysed individually. In contrast to the 1997 results, the variability for the independent grabs was rather high (20-120%), even for a compound such as toluene which was present at relatively high concentrations.

Table 3.2.3: Average VOC concentrations and standard deviations (n=8) in water (C_w), salinity (S), and temperature (T) at selected sampling stations.

Sample	C_w (ng/l)	S (g/l)	T (°C)	log K_{ow}	log $K_{oc,sw}$	log $K_{oc,(S=0)}$	log $K_{oc,sw,eq}$
CHCl3/S22	300 ± 70	1.45	11.4	1.93	1.81	1.81	1.36
/435	19 ± 9	35	11.0		3.64	3.55	
CCl4/S18	12 ± 13	5.38	11.6	2.73	2.04	2.02	1.67
DCE11/S18	21 ± 7	5.38	11.6	1.79	1.10	1.08	0.96
/S04	24 ± 19	24.7	10.2	1.47	1.65	1.60	
/S12	80 ± 61	11.0	11.4		0.60	0.58	
/S15	100 ± 65	8.65	11.8		1.10	1.08	
/S18	158 ± 77	5.38	11.6		0.26	0.24	
/S22	63 ± 21	1.45	11.4		1.67	1.67	
TRI/S07	12 ± 6	20.8	10.2	2.48	1.85	1.76	1.5
/S15	84 ± 55	8.65	11.8		2.14	2.10	
/S18	163 ± 107	5.38	11.6		0.60	0.57	
/S22	266 ± 212	1.45	11.4		0.78	0.77	
TCE/S07	18 ± 21	20.8	10.2	2.42	2.34	2.26	1.58
/S15	67 ± 45	8.65	11.8		1.99	1.96	
/S18	134 ± 66	5.38	11.6		0.94	0.92	
/S22	222 ± 60	1.45	11.4		1.83	1.82	
TTCE/S01	5.0 ± 3.5	29.5	10.2	2.88	3.53	3.39	2.14
/800	6.0 ± 7.5	35	10.9		3.97	3.81	
BENZ/S07	70 ± 114	20.8	10.2	2.13	2.55	2.47	
/S09	36 ± 39	16.4	10.7		3.02	2.97	
/S12	58 ± 72	11.3	11.4		2.21	2.17	
/S15	55 ± 64	8.65	11.8		2.38	2.35	
/S18	39 ± 53	5.38	11.6		2.11	2.09	
/S22	102 ± 240	1.45	11.4		2.40	2.39	
TOL/S07	29 ± 13	20.8	10.2	2.69	3.03	2.96	1.6
/S12	71 ± 58	11.0	11.4		2.32	2.28	
/S15	66 ± 64	8.65	11.8		2.99	2.96	
/S18	64 ± 35	5.38	11.6		2.37	2.35	
/S22	56 ± 43	1.45	11.4		3.23	3.22	
ETBEN/S12	35 ± 34	11.0	11.4	3.15	2.16	2.11	2.03
/S15	32 ± 39	8.65	11.8		2.36	2.32	
/S18	23 ± 17	5.38	11.6		1.81	1.78	
MPXYL/S04	33 ± 38	24.7	10.2	3.19	2.61	2.51	
/S15	37 ± 37	8.65	11.8		2.47	2.44	
/S18	34 ± 41	5.38	11.6		1.89	1.87	
OXYL/S01	15 ± 9	29.5	10.2	3.12	2.16	2.02	2.02
/S04	22 ± 13	24.7	10.2		2.62	2.50	
/S15	31 ± 30	8.65	11.8		2.25	2.21	
/S18	40 ± 52	5.38	11.6	±	1.52	1.49	

Log K_{ow}, logarithm of the calculated in situ partitioning coefficient; log $K_{oc/sw}$, logarithm of the calculated *in situ* partitioning coefficient at zero salinity; log $K_{oc,(S=0)}$ and logarithm of the equilibrium partitioning coefficient for salt water log $K_{oc,eq}$ [9].

The concentrations of VOCs found in sediment can be related to those observed in the water column. In order to study this, the *in situ* sediment/water partitioning of VOCs was compared with equilibrium partitioning. In 40 cases, concentrations of VOCs in the sediment layer were above the limit of detection during this study (Table 3.2.2). At the same sites VOC concentrations in the water column were determined in the period 1994-1997, as described in previous papers [4,6]. Average water column concentrations for these sites are presented in Table 3.2.3. From the experimental sediment layer concentration ($C_{s,meas}$), the organic carbon fraction of the sediment (f_{oc}), and the water concentration ($C_{w,meas}$) $K_{oc/sw}$ values were calculated (Table 3.2.3). $K_{oc/sw}$, the *in situ* partitioning coefficient of VOCs between the organic carbon fraction of the sediment and salt water, is defined as

$$K_{oc,sw} = \frac{C_{oc,meas}}{C_{w,meas}} = \frac{C_{s,meas}}{C_{w,meas}} . f_{oc} \qquad (1)$$

with $C_{oc,meas}$ the experimentally determined concentration of VOCs in the organic carbon fraction of the sediment. Indeed, given that VOCs in sediment are mainly associated with the organic fraction of the sediment, concentrations of VOCs in sediment can be expressed on the basis of the organic carbon fraction.

In the literature *in situ* partitioning coefficients are often compared with K_{ow} (octanol/water equilibrium partitioning coefficient) values [29]. However for the marine environment the *in situ* partition coefficient $K_{oc/sw}$ cannot be immediately compared with K_{ow} because of salinity. Indeed, K_{ow} represents the partition behaviour between an organic phase (octanol) and (deionised) water, whereas $K_{oc/sw}$ represents a partitioning process between an organic phase and salt water. Dewulf *et al.* [9] have shown that $K_{oc,sw}$ can be converted into a partitioning coefficient $K_{oc,(S=0)}$, reflecting the partitioning between organic carbon and deionised water by means of: $K_{oc,(S=0)} = K_{oc/sw} . (H/H_{sw})$ where H and H_{sw} are the dimension-less Henry's law coefficients of the compound of interest for deionized and salt water, respectively. By considering the average salinities and temperatures at the different locations, and data for H and H_{sw} as a function of temperature and salinity from Dewulf *et al.* [9], the *in situ* partitioning coefficient at zero salinity $K_{oc,(S=0)}$, can be calculated and the results are given in Table 3.2.3. In Figure 3.2.3

$K_{oc, (S=0)}$ is plotted in function of K_{ow}. It is obvious that there is a large scatter in the data points, which cannot be attributed to the rather large number of measurements close to the LOD. Even so, linear regression shows a positive relation between log $K_{oc, (S=0)}$ and log K_{ow} data with a slope of 0.58 and an intercept of 0.64 with r = 0.39 (n = 40), which is significant at $\alpha = 0.05$ (P = 0.012). In other words, although the scatter is large, the log K_{ow} data gives an indication of the *in situ* partitioning behaviour of the volatile organic compounds. The slope, with a value lower than unity, suggests that there is a difference in polarity between the organic matter in the sediment and octanol.

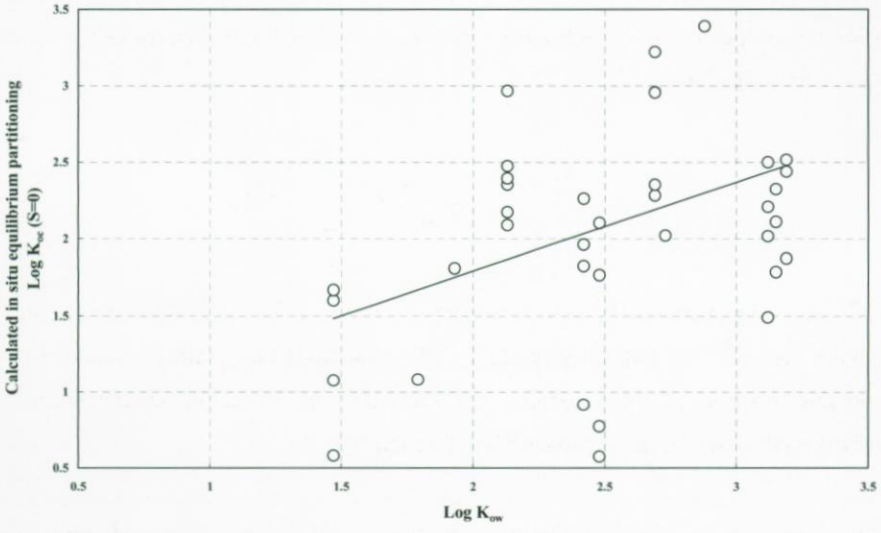

Figure 3.2.3: Relationship between K_{ow} (saltwater) and the *in situ* partitioning coefficient K_{oc} (S=0), determined with the present data set. (S = salinity)

In a second step, the *in situ* partitioning coefficients can be compared with experimental equilibrium partitioning coefficients in order to establish whether *in situ* partitioning is in equilibrium or disequilibrium. In a previous study experimental equilibrium partitioning coefficients of compounds of interest between organic matter in sea sediment and (deionized) water, $K_{oc,eq}$, were determined by Dewulf *et al.* [9] during their study of the sorption of VOCs onto marine sediments by using a miscible displacement technique. As before, the values were extrapolated to zero salinity to compensate for differences in salinity (e.g. Scheldt river versus North Sea). In Figure 3.2.4, the log $K_{oc, (S=0)}$ data are compared with experimental equilibrium partitioning coefficients at zero salinity ($K_{oc,eq,}$

$_{(S=0)}$). Linear regression now shows a slope of 0.89 and an intercept of 0.63 with r = 0.33. Although the linear regression is not significant at $\alpha = 0.05$ (P = 0.076), the value of the slope shows that the sorption behaviour of VOCs onto marine sediments as predicted from laboratory equilibrium partitioning experiments (cf. above) can be used to estimate the *in situ* partitioning behaviour.

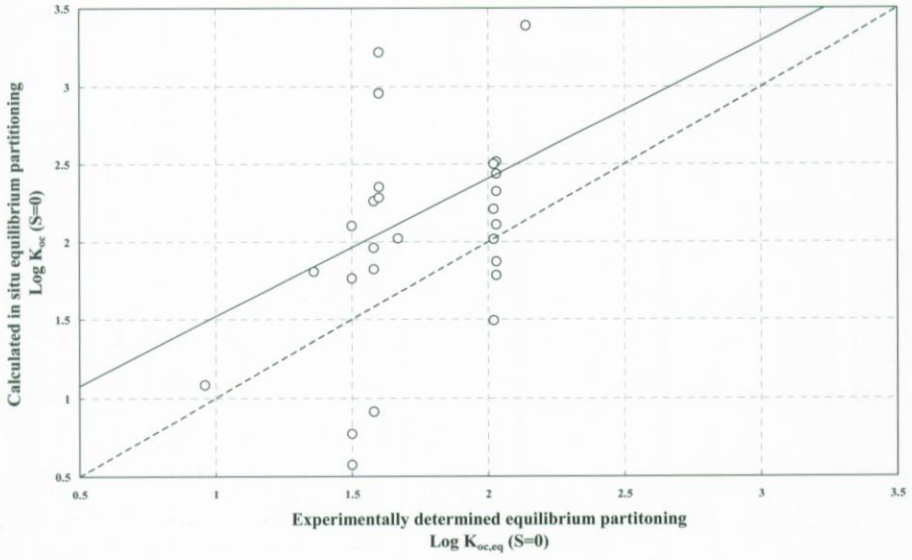

Figure 3.2.4: Relationship between the equilibrium partitioning coefficient $K_{oc,eq}$ (S=0), as determined by Dewulf *et al.* [9] and the *in situ* partitioning coefficient K_{oc}, determined with the present data set. (S= salinity)

The role of the sediment layer as a sink or source for VOCs can be assessed by comparing its role in partitioning with that of the water body and the atmosphere. From mass balances and equilibrium partitioning coefficients the fraction of a VOC in the sediment layer at equilibrium partitioning can be calculated from

$$M = C_{w,eq}.V_w+C_{s,eq}.V_s+C_{a,eq}.V_a, \qquad (2)$$

with M the total mass of a given VOC in the marine system, $C_{w,eq}$, $C_{s,eq}$, and $C_{a,eq}$ the concentrations at equilibrium in water, sediment and air, respectively, and V_w, V_s and V_a the volumes of the compartments water, sediment and air. With $H = C_{a,eq}/C_{w,eq}$ and K_{eq} =

$C_{s,eq}/C_{w,eq}$, with K_{eq} the equilibrium partitioning coefficient between sediment and water, the mass fractions of the VOCs at equilibrium in the water ($f_{w,eq}$), air ($f_{a,eq}$) and sediment ($f_{s,eq}$) compartments can be calculated from, respectively,

$$\frac{1}{f_{w,eq}} = 1 + K_{eq}.\frac{V_s}{V_w} + H.\frac{V_a}{V_w} \quad (3)$$

$$\frac{1}{f_{a,eq}} = 1 + \frac{V_w}{H.V_a} + \frac{K_{eq}.V_s}{H.V_a} \quad (4)$$

$$\frac{1}{f_{s,eq}} = 1 + \frac{V_w}{K_{eq}.V_s} + H.\frac{V_a}{K_{eq}.V_s} \quad (5)$$

K_{eq} can be calculated from $K_{eq} = \gamma.f_{oc}.K_{oc/sw,eq} + \theta$ with γ the apparent density of the sediment and θ the porosity of the sediment [9] and Henry's law coefficient is known from Dewulf *et al.* [30]. Considering an area of 1 km² and the same sediment and atmospheric heights as in the fugacity model of Mackay [31] and Mackay and Paterson [8,32,33] (1 cm and 2 km, respectively), and taking into account the depth of the water column at the sampling locations (30, 9 and 10.4 m for sampling locations 800, S15 and S22, respectively), the three fractions of interest can be calculated from equations 3 - 5. The results of these calculations are presented for tetrachloroethene in Figure 3.2.5. Tetrachloroethene was selected, because it is the only VOC for which measurable levels were found both in the sediment layer of the North Sea and of the Scheldt estuary.

Alternatively, the *in situ* partitioning can be studied by considering the VOC concentrations found in the sediment layer, the atmosphere and the water column. By substituting the actual concentrations of a VOC in water, sediment and air in Eq. 2, one finds

$$M = C_{w,meas}.V_w + C_{s,meas}.V_s + C_{a,meas}.V_a \quad (6)$$

with $C_{w,meas}$ and $C_{s,meas}$ as before and $C_{a,meas}$ the concentration measured in air. The mass fractions in each compartment can then be calculated by multiplying the concentration in each of these compartments with that compartment's volume, and dividing the outcome by the total mass of the VOC. The fractions were calculated using the sediment

concentrations from this work and the air and water column concentrations from previous work [6], and using the same volumes of the three compartments as above; the results are presented in Figure 3.2.5.

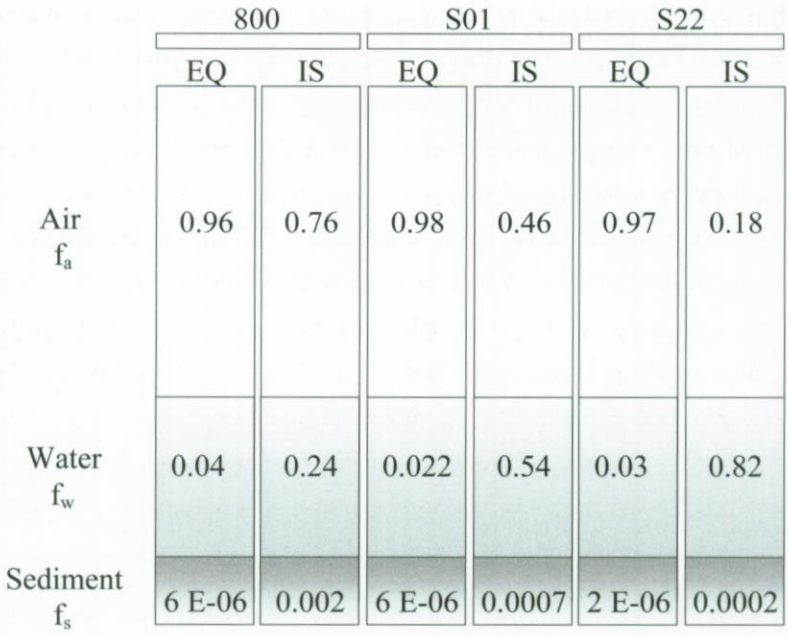

Figure 3.2.5: Comparison of the calculated fractions of tetrachloroethene in air, water and sediment at equilibrium (EQ) and *in situ* (IS) at the sampling locations 800, S01 and S22.

3.2.4 Discussion

After minor modification, the method used for the determination of VOCs in marine organisms [28] proved to be equally successful for the determination of VOCs in marine sediments. The repeatability, recoveries and LODs reported in this paper are similar to those reported in the literature (Table 3.2.1). The repeatability of the current method averaged around 15%, which is fully satisfactory compared to what is reported for similar P&T techniques (1-30%) [10,11]. With the current method the LODs ranged from 4 to 200 ppt, depending on the background concentrations and the characteristics of the analytes. Al Rekabi *et al.* [11] reported LODs between 40 and 50 ppt and Bianchi *et al.* [10] obtained LODs ranging from 20 to 30 ppt using P&T. The analyte recovery generally was above 80%. On the one hand, this is to be expected because a sandy sediment was

used which will not adsorb the VOCs as strongly as a sediment with a large clay or organic fraction. On the other hand, Charles and Simmons [24] found that neither sediment composition nor sample weight influenced the outcome of a P&T analysis. In any case, the choice of a sandy sediment for this study was deliberate. Most sediments we had to analyse were of a sandy nature and losses due to volatilisation were considered to be the most prominent danger [19]. Bianchi *et al.* [10] found comparable results when using the same approach as in our study, i.e. long purge times, a relatively high purge temperature and a minimum of sample handling. Most authors report special measures to minimise losses during sampling and storage, but the effect of these measures is hardly ever discussed. Siegrist and Jenssen [15] discussed the effects of several sampling methods on the determination of VOCs in contaminated soil in detail. The highest recoveries were obtained when the sample container was immersed in methanol immediately after sampling. Container headspace volume and soil disturbance contributed less to what they called negative bias (i.e. measured value lower than actual). For the present work, a zero headspace volume and an additional sealing with Teflon tape was applied to minimise losses. The analyte recoveries of over 90% obtained after storage of a spiked sandy sediment sample certainly illustrate the adequacy of these measures.

The results of the environmental analyses show that VOC concentrations are below the detection limits at nearly all sampling stations with the exception of those in the Antwerp harbour area (Table 3.2.2). At a first glance, this is somewhat surprising because the river Scheldt is regarded as being a heavily polluted stream and the major source of contamination of the Belgian coastal waters [7]. However, sediments are not widely regarded as a major source or sink of VOCs. As K_{ow} is low for most VOCs, significant sorption is not expected [3,9]. The present experimental results seem to support this thesis. A positive relation was found between log $K_{oc,\,(S=0)}$, determined *in situ*, and log K_{ow} (Figure 3.2.3). This indicates that the *in situ* partitioning behaviour of the volatile organic compounds can be predicted from their K_{ow}. The lower-than-unity slope suggests a polarity difference between octanol and the organic carbon fraction of the sediment, in the sense that VOCs apparently have a lower affinity for the organic carbon fraction of sediment than for octanol.

No significant relationship between $K_{oc,(S=0)}$ and $K_{oc,eq,(S=0)}$ could be demonstrated at α = 0.05 (P = 0.076) (Figure 3.2.4). The value of the slope suggests that the sorption

behaviour of VOCs onto marine sediments as predicted from laboratory equilibrium partitioning experiments can be used to estimate the *in situ* partitioning behaviour. However, the regression line is found above the bisector. This suggests that the sediment layer is 'oversaturated' by VOCs when compared to the aqueous layer. In other words, the sediment layer may act as a source of VOCs. The latter can be studied in more detail by using the model of Dewulf [34], who developed a dynamic exchange model for VOCs in the North Sea and the Scheldt estuary and estimated that only 0.0006% of the total VOC burden is present in the sediment fraction. This conclusion is confirmed when the mass fractions of tetrachloroethene in air, water and sediment are calculated according to this model (Figure 3.2.5). The results indeed show that the role of the sediment as a sink is of minor importance. However, when calculating the mass fractions of tetrachloroethene based on the *in situ* concentrations [34], the *in situ* partitioning into the sediment layer and, especially, the water column is higher than expected from equilibrium partitioning calculations (Figure 3.2.5). This may signify that there are additional sources in the sediment or in the water column. Additional sources are highly likely in the Scheldt estuary and can be attributed to anthropogenic activities along the river. However, even for the more remote sampling location 800, the role of the sediment layer and water body are underestimated. Direct anthropogenic inputs, as in the Scheldt estuary, are rather unlikely for this location. However, several alternatives can be suggested. Firstly, long-range aqueous transport from riverine inputs discharged into the North Sea can explain these relatively high water and sediment concentrations. Secondly, in the literature a number of biogenic marine sources have been mentioned for tetrachloroethylene [4]. Finally, the history of the sediment may play a role. Finally, but less likely, the higher (local) anthropogenic emissions in marine waters may have led to a relatively high accumulation in the sediment layer, from which the VOCs are, subsequently slowly, released.

Finally, although the current findings allow suggesting that the marine environment as a whole, and marine organisms in particular, are not threatened by the presence of VOCs in sediment, some caution is warranted. The results show that VOCs are mainly associated with the organic carbon fraction of the sediment. Considering that this fraction is primarily associated with the fine fractions of sediments, it should be noted that the concentrations of VOCs normalised for the fine-fraction content of sediments are similar to those of contaminants such as PCBs [35]. The fine fraction is, in addition, the most

important one for organisms. Many conveyer belt species or funnel feeders prefer ingesting and reworking the finer fraction of sediments. Contaminated-deposit-feeding organisms may significantly contribute to the dietary uptake of toxic chemicals by demersal fish, which will result in a food web transfer [36]. In other words, VOCs in sediment could contribute to, or be a main source of, VOC levels found in fish and higher organisms.

3.2.5 Conclusions

The current analytical methodology allows the determination of VOCs in marine and estuarine sediments with an acceptable recovery and reproducibility. Although the VOC levels in many sediments are at or below the detection limits, improving the detection limit is not urgently required. The current study illustrates that the sorption behaviour of VOCs in sediments, determined by laboratory experiments, can be used to estimate their behaviour under environmental conditions. Because of this, it can be assumed that the concentrations in marine sediments will be low and that, in general, VOCs in sediments should not be regarded as a major problem in the marine environment. However, the present study also shows that local situations cannot solely be explained by an equilibrium partitioning approach and that local high concentrations may be a cause for concern, especially with regard to organisms.

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3.3 Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium)[‡‡]

Summary

Twenty eel from various inland water bodies in Flanders (Belgium) were analysed for a total of 52 VOCs. The most prominent VOCs are the BTEX and a number of chlorinated compounds such as chloroform and tetrachloroethene. The observed levels could be linked to the major emission sources and the present study gives new evidence that combustion of fossil fuels is a major source of BTEX in the environment. The concentrations in eel seem to be a reflection of the actual concentrations in their environment. For fish from the same location similar patterns and concentrations were observed, and the concentrations agree with what can be expected from those of the water column. Generally speaking, the observed concentrations do not seem to pose a threat for organisms. More definite statements will, however, require a larger dataset. The study suggests that yellow eel can possibly be used as a biomonitor or sentinel organism for VOCs.

[‡‡] From *J. Environ. Monit.*, 5 (2003) 876-884.

3.3.1 Introduction

Volatile organic compounds (VOCs) are well-known atmospheric contaminants that are frequently determined in air, drinking water, fresh water, effluents and soils [1-3]. Most representatives of the group are important industrial compounds with a high annual production [4] which can be anywhere in the range from several hundred thousand tonnes for e.g. tetrachloromethane, to more than 10 billion tonnes for benzene [5,6]. In Belgium, the emissions of the chlorinated hydrocarbons (CHCs) chloroform, 1,1,1-trichloroethane, and tri- and tetrachloroethene, exceed those of *e.g.* lead, lindane and atrazine [7]. Moreover, benzene, toluene, ethylbenzene and the xylenes (BTEX) are important additives to unleaded gasoline and are present in crude oil. Several international organizations therefore regard VOCs as compounds with a high research priority [8,9].

The low values of the logarithm of the octanol-water partition coefficients (log K_{ow}) of the VOCs, typically, 1 – 2, led to the general belief that bioconcentration should be considered insignificant [5,10]. As a result, the presence of VOCs in organisms was studied by a limited number of research groups only and there are few recent findings in the literature [11]. The considerable analytical problems associated with the determination of these compounds in environmental matrices, specifically in biota, can be regarded as another reason for the lack of information. It was somewhat surprising, therefore, that recent studies showed the general presence of a number of important VOCs in the tissue of marine organisms from different levels of the food chain [11]. It was also found that the concentrations in marine organisms were up to a thousand times higher than those in the surrounding water. The bioconcentration factors calculated from these data were generally higher than those reported in the literature. A possible explanation is the continuous exposure of organisms to low or even undetectable levels of these compounds in the water column. Determination in the water column alone is, therefore, insufficient.

Aquatic organisms can, and have been, used successfully to monitor contaminants in various ecosystems, especially when the concentrations of these compounds in the water column are extremely low [12]. For an organism to become a potential biomonitor or sentinel organism, several criteria should be fulfilled. First and foremost, the organism should reflect the actual condition of the surrounding water column. This implies that it should show little or no migratory behaviour and that the species should commonly occur in the area under investigation. The yellow eel, *Anguilla anguilla* L., appears to be a most

adequate indicator organism for the pollution status of freshwater environments. Eels are benthic fish which have a widespread geographical distribution. They are carnivorous organisms that predate mainly on insect larvae, worms, crustaceae, snails, mussels and fish, in particular small bottom-dwelling species. Moreover, yellow eel has a high proportion of lipids in its body, which facilitates the accumulation of lipophilic contaminants. The accumulation is further promoted by the fact that no spawning occurs during the eels' stay in inland waters. Eel is also essentially sedentary and normally does not migrate [12]. The same authors showed that yellow eel reflects rapid changes in the concentrations of organic contaminants in the surrounding water.

In this study, a limited number of eel, which were sampled as part of a routine monitoring programme, were analysed by means of a previously developed method [13] for their VOC content. The study is intended as a screening exercise to get an impression of the concentrations of VOCs in yellow eel, the potential environmental hazard and the possibility of the future use of yellow eel as an indicator organism.

3.3.2 Materials and experimental procedures

Samples and sampling

Eels were sampled by means of either electrofishing along riverbanks, fyke fishing or seine netting. Samples were initially collected in the framework of the fish stock assessment programme of the Institute for Forestry and Game Management, which aims at monitoring fish and the biotic integrity of riverine and lacustrine waters all over Flanders. The samples were subsequently analysed for their PCB, organochlorine pesticide and heavy metal content, and in the framework of this study, for the presence of VOCs. Because of the limited budget and, consequently, the limited number of eel that could be analysed, a compromise had to be reached: we decided to select samples for VOC analysis covering a large geographical area rather than study only a few sites in some more detail. Pooling was also not possible because the analysis can only be done on individual eel samples [13]. An overview of the sixteen inland water stations is given in Table 3.3.1. The stations can be characterized as rivers (>10 m width, 4 stations), brooks (< 10 m width, 2 stations), canals (8 stations) and enclosed water bodies such as ponds (6 stations). They are located in rural as well as in densely populated industrial areas (Fig. 3.3.1). Twenty eels were selected from the 30-70 cm size range (Table 3.3.1).

Samples were wrapped in aluminium foil and stored at -28°C in an airtight freezer located in a solvent-free area.

Lipids were measured by total lipid extraction following the method of Bligh and Dyer [14].

Figure 3.3.1: Sampling locations in the region of Flanders (Belgium) (Source: OC Gis Vlaanderen and AMINAL, Water Section; see also Table 1).

Analytical methodology

A detailed description of the analytical methodology is given elsewhere [13,15]. Briefly, biological tissue is first homogenised at 0°C in an ultra-turrax blender and transferred to a 40-ml vial. After addition of 25 ml of water and the internal standard (1,1,1-trifluorotoluene), the homogenate is treated for 20 min at 0°C in an ultrasonic bath to further disrupt the tissue. The glass vessel is then connected to a Tekmar (Cincinnati, OH, USA) LSC 2000 purge-and-trap apparatus coupled to a Finnigan Magnum (Finnigan, San José, CA, USA) gas chromatograph-mass spectrometer (GC-MS). The volatiles are forced out of the tissue by purging with a stream of helium while heating at 70°C, and trapped onto a Vocarb 4000 sorbent trap (Supelco, Bellefonte, PA, USA). After purging, the trap is backflushed while being rapidly heated to 250°C, the analytes are desorbed and, next,

Table 3.3.1: Overview of sampling stations* and sampled eel.

No	Location	Type of water	Surroundings	River basin	Length (cm)	Weight (g)	Lipid content (%)
1	Leie, Menen	River	Industrial	Leie	65	467	33
2	Albertkanaal, Langerlo	Canal	Industrial	Demer	67	616	31
3	Kanaal van Leuven to the Dijle, Tildonk	Canal	Industrial	Dijle-Zenne	57	390	30
4	Groot Zuunbekken, St.-Pieters-Leeuw	Pond	Industrial	Dijle-Zenne	55	321	9
5	Kanaal van Leuven to the Dijle, Tildonk	Canal	Industrial	Dijle-Zenne	50	251	33
6	Grensmaas, Molensteen	River	Rural	Maas	67	601	26
7	Oude Leie Ooigem	Pond	Rural	Leie	62	411	24
8	Witte Nete, Dessel	River	Rural	Nete	52	281	16
9	Pond at Rijksdomein, Hofstade	Pond	Rural	Dijle-Zenne	65	625	29
10	Grensmaas, Molensteen	River	Rural	Maas	57	365	23
11	Zandwinningsput, Weerde	Pond	Industrial	Dijle-Zenne	60	385	25
12	Albertkanaal, Langerlo	Canal	Industrial	Demer	37	539	33
13	A, Poppel	Brook	Rural	Maas	45	177	16
14	Kanaal Bocholt-Herentals, Blekerheide	Canal	Industrial	Maas	51	262	30
15	Oude Leie, Wevelgem	Pond	Industrial	Leie	57	307	25
16	Putten van Niel, Niel	Pond	Industrial	Benedenschelde	45	181	20
17	Kanaal Bocholt-Herentals, Sluis Herentals	Canal	Industrial	Nete	50	262	24
18	Warmbeek, Achel	Brook	Rural	Maas	53	277	16
19	Darse, Vilvoorde	Canal	Industrial	Dijle-Zenne	47	191	31
20	Kanaal Beverlo, Leopoldsburg	Canal	Industrial	Nete	59	321	21

*Also see Fig. 3.3.1

trapped in a cryofocusing module (-120°C) connected to the GC column (J&W, Folsom, CA, USA, DB-VRX, 60 m, 0.25 mm id, 1.4 µm film).

The analytes were injected into the column by rapidly heating the module from -120°C to 200°C in 0.75 min. Temperature programming of the GC and data acquisition were started simultaneously. The temperature of the GC oven was held at 35°C for 6 min and then linearly increased to 200°C at 4°C/min. This temperature was then held for 4 min. Helium with an inlet pressure of 16 psi was used as the carrier gas. The ion-trap detector was operated in the electron ionisation (EI) mode with the multiplier voltage set at 2400 V, the axial modulation (A/M) amplitude at 3.5 V and the emission current at 12 µA. The manifold temperature was set at 220 ° C. The mass range was 50-250 amu and the scan rate, 1000 ms. The filament delay was 180 s, and a mass defect of 50 mmass / 100 amu and a background mass of 55 amu were selected.

VOC concentrations are expressed on a wet weight basis throughout the paper.

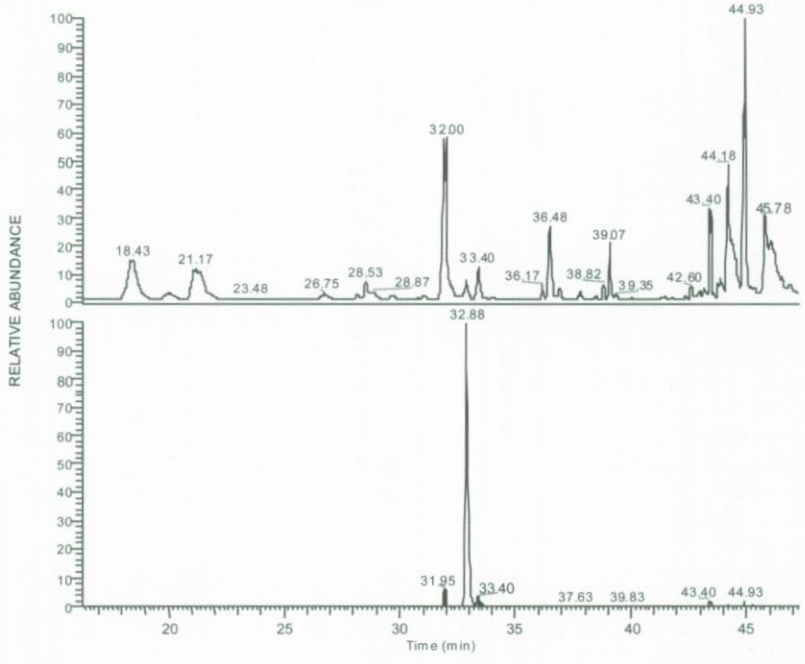

Figure 3.3.2: Total ion count GC-MS chromatogram for eel sample No. 5 and extracted ion chromatogram (*m/z* 164+165) for tetrachloroethene (bottom).

Table 3.3.2: Set of 52 VOCs studied and relevant analytical information.

Sequence number	Compound	Masses[1] (m/z)	Retention time (min)	LOD[2] (ng/g)
1	*trans*-1,2-Dichloroethene	61/96/98	2:24	0.1
2	1,1-Dichloroethane	63/83/97	3:26	0.1
3	*cis*-1,2-Dichloroethene	61/96/98	6:04	0.1
4	2,2-Dichloropropane	77/79/97	7:14	0.1
5	Bromochloromethane	130/128/49	6:56	0.1
6	Chloroform	83/85	7:17	0.3
7	1,1,1-Trichloroethane	97/61/99	11:40	0.05
8	Tetrachloromethane	117/119	14:24	0.1
9	Dichloropropene	39/110/77	13:20	0.2
10	Benzene	78	15:04	0.2
11	1,2-Dichloroethane	62/64	11:12	0.01
12	Trichloroethene	130/95/60	20:34	0.5
13	1,2-Dichloropropane	62/63/76	19:45	0.2
14	Dibromomethane	174/172/93	19:57	0.5
15	Bromodichloromethane	83/85/47	20:53	0.4
I.S.	Trifluorotoluene[3]	146/127/96	23:00	-
16	*cis*-1,3-Dichloropropene	75/110/39	25:14	0.05
17	Toluene	91	29:22	0.4
18	*trans*-1,3-Dichloropropene	75/110/39	27:49	0.1
19	1,1,2-Trichloroethane	97/61/99	28:21	0.01
20	Tetrachloroethene	166/129/94	32:33	0.1
21	1,3-Dichloropropane	76/78/41	29:38	0.05
22	Dibromochloromethane	129/127/48	30:28	0.05
23	1,2-Dibromoethane	107/109/27	31:34	0.05
24	Chlorobenzene	112/114/77	35:30	0.1
25	1,1,1,2-T-etrachloroethane	131/133/95/122	35:14	0.02
26	Ethylbenzene	91/105/106	36:36	0.1
27	*m*-Xylene	91/105/106	37:30	0.2
28	*p*-Xylene	91/105/106	37:30	0.2
29	*o*-Xylene	91/105/106	39:02	0.2
30	Styrene	103/78/51	38:48	0.05
31	Bromoform	173/171/175	37:22	0.05
32	Isopropylbenzene	105/120/77	40:34	0.1
33	1,1,2,2-Tetrachloroethane	83/101/131	38:57	0.1
34	Bromobenzene	158/156/77	41:11	0.1
35	1,2,3-Trichloropropane	75/110/39	39:32	0.3
36	*n*-Propylbenzene	91/105/120	42:18	0.3
37	2-Chlorotoluene	91/126	42:28	0.1
38	1,3,5-Trimethylbenzene	105/120/77	43:35	0.1
39	4-Chlorotoluene	91/126	42:49	0.05
40	*tert*-Butylbenzene	91/119	44:31	0.05
41	1,2,4-Trimethylbenzene	105/77/120	45:01	0.3
42	*sec*-Butylbenzene	134/105	45:21	0.2
43	1,3-Dichlorobenzene	146/111/75	45:25	0.2
44	*p*-Isopropyltoluene	119/91/39	46:10	0.1
45	1,4-Dichlorobenzene	146/111/75	45:41	0.1
46	*n*-Butylbenzene	91/134	47:42	-[4]
47	1,2-Dichlorobenzene	146/111/75	46:58	0.05
48	1,2-Dibromo-3-chloropropane	157/75/57	48:44	0.05
49	1,2,4-Trichlorobenzene	180/145/109	53:55	6
50	Hexachlorobutadiene	260/225/190	55:10	0.4
51	Naphthalene	128/102	54:48	4
52	1,2,3-Trichlorobenzene	180/145/109	55:33	6

[1] In order of relative abundance, [2] For a 40-g sample with extracted ions, [3] Internal standard, [4] not determined

3.3.3 Results and Discussion

VOC concentrations in eel

The twenty eel from the various inland water bodies were analysed for a total of 52 VOCs which are listed in Table 3.3.2. Compounds were identified on the basis of their mass spectrum and their concentrations were calculated by using at least two selected ion masses (exceptions: benzene and toluene). As an illustration, a full scan GC-MS chromatogram and a selected ion chromatogram for tetrachloroethene in eel sample No. 5 are shown in Fig. 3.3.2. Detection limits (LODs) in the selected-ion mode for 40 g samples were calculated on the basis of a signal-to-noise ratio of 3 or 3 times the standard deviation of the blank. They varied between 0.01 ng/g wet weight (1,2-dichoroethane, 1,1-dichloroethane and tetrachloromethane) and 6 ng/g wet weight (trichlorobenzene) depending on the background levels and the amount of sample.

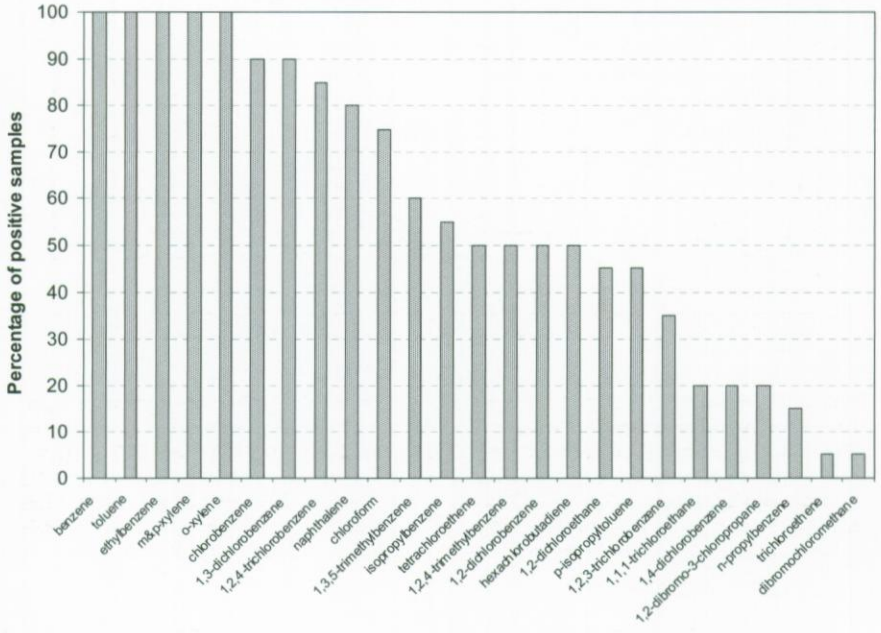

Figure 3.3.3: Percentage of positive samples for the detected VOCs in order of abundance.

All relevant data are presented in Table 3.3.3. The results show that about half of the target VOCs, *i.e.* 25 out of 52, were detected in one or more eel samples. A detailed breakdown of the results is presented in Fig. 3.3.3 which shows the percentage of samples that was positive for a given VOC. One striking observation is that the BTEX compounds

were present in all samples. A further five compounds, chlorobenzene, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, naphthalene and chloroform, were present in 70-90% of all samples, and a 35-60% positive score was obtained for nine VOCs, 1,3,5-trimethylbenzene, isopropylbenzene, tetrachloroethene, 1,2,4-trimethylbenzene, 1,2-dichlorobenzene, hexachlorobutadiene, 1,2-dichloroethane, p-isopropyltoluene and 1,2,3 trichlorobenzene. The other VOCs were found in 20% of the samples or less.

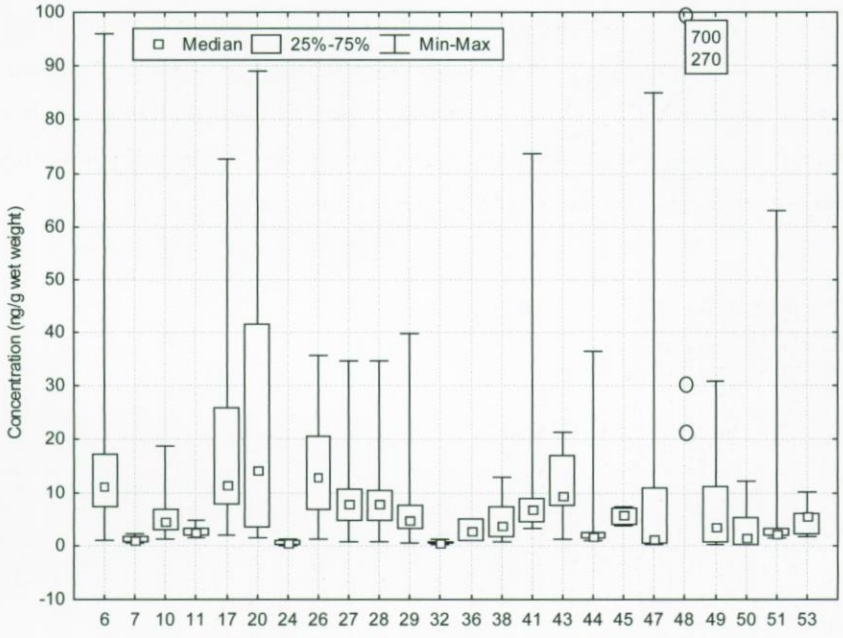

Figure 4: Box and whisker plot of the detected VOCs for all eel samples, with from left to right: (6) chloroform; (7) 1,1,1-trichloroethane; (10) benzene; (11) 1,2-dichloroethane; (17) toluene; (20) tetrachloroethene; (24) chlorobenzene; (26) ethylbenzene; (27) m-xylene; (28) p-xylene; (29) o-xylene; (32) isopropylbenzene; (36) n-propylbenzene; (38) 1,3,5-trimethylbenzene; (41) 1,2,4-trimethylbenzene; (43) 1,3-dichlorobenzene; (44) p-isopropyltoluene; (45) 1,4-dichlorobenzene; (47) 1,2-dichlorobenzene; (48) 1,2-dibromo-3-chloropropane; (49) 1,2,4-trichlorobenzene; (50) hexachlorobutadiene; (51) naphthalene; (53) 1,2,3-trichlorobenzene.

The concentrations of the VOCs that were detected varied considerably, as is graphically illustrated by the box and whisker plot of Fig. 3.3.4. The median concentrations typically were 1-10 ng/g, ranging from 0.5 ng/g for isopropylbenzene to 14 ng/g wet weight for tetrachloroethene. High concentrations of over 30 ng/g were found for twelve of the VOCs, with a staggering 700 ng/g wet weight for 1,2-dibromo-3-chloropropane in eel from the Albertkanaal, Langerlo, as the maximum. Extensive statistical testing, such as

Table 3.3.3: Concentrations (ng/g ww) of VOCs detected in freshwater eel [1]

VOC	Sampling stations[2]																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Chloroform	15	9.4	17	96	30	2.9	3.9	-	-	11	9.7	7.4	1.0	-	-	10	16	-	13	23
1,1,1-Trichloroethane	2.2	-	-	-	-	-	-	-	-	-	-	0.5	-	1.5	-	-	0.7	-	-	-
Benzene	2.6	2.2	7.0	19	10	3.1	2.7	11	4.9	6.9	4.8	3.5	1.2	8.9	6.0	3.9	4.2	1.7	4.2	6.6
1,2-Dichloroethane	-	1.8	-	-	-	-	-	-	2.5	3.3	3.5	2.4	-	4.9	-	1.4	2.0	-	2.0	-
Toluene	10	5.2	33	73	47	7.4	6.7	41	13	20	13	12	1.9	22	11	11	11	3.7	8.5	30
Tetrachloroethene	64	11	42	1.5	89	2.0	-	-	-	3.6	-	18	-	31	6.2	-	-	-	-	-
Chlorobenzene	0.3	0.3	0.5	-	1.3	0.3	0.2	0.3	0.6	1.1	0.9	0.5	0.1	0.6	0.8	0.6	0.9	-	0.7	1.1
Ethylbenzene	5.7	5.7	13	21	36	7.9	4.9	10	15	30	20	14	1.2	18	12	12	24	5.8	13	29
m&p-Xylene	7.8	3.1	8.9	35	18	4.0	3.0	8.6	7.8	13	8.2	7.1	0.7	11	6.9	6.2	9.7	2.4	5.5	15
o-Xylene	5.9	2.2	6.6	40	12	2.9	2.1	9.2	4.3	7.1	4.5	4.8	0.6	8.3	4.7	4.1	5.8	1.6	3.6	11
Isopropylbenzene	0.5	0.2	0.5	1.2	0.5	0.2	-	-	-	-	-	-	-	0.7	0.5	0.8	0.5	-	0.4	-
n-Propylbenzene	-	-	-	5.0	-	-	-	-	-	-	-	-	-	-	-	1.0	2.8	-	-	-
1,3,5-Trimethylbenzene	7.9	5.4	9.3	13	-	1.2	-	-	-	-	-	-	-	3.6	6.9	1.7	2.5	0.7	1.6	3.9
1,2,4-Trimethylbenzene	8.8	3.4	4.6	74	-	-	-	-	-	-	-	-	-	7.1	14	6.7	9.0	3.3	5.4	-
1,3-Dichlorobenzene	5.1	7.7	18	-	-	1.2	7.9	17	11	11	8.3	8.4	3.9	18	18	17	10	5.8	8.4	21
p-Isopropyltoluene	-	-	-	-	-	-	-	-	-	-	1.7	1.7	1.0	-	2.5	0.9	2.7	1.5	1.7	36
1,4-Dichlorobenzene	6.9	3.7	4.6	-	-	-	-	-	-	-	-	-	-	-	-	-	7.5	-	-	-
1,2-Dichlorobenzene	41	7.7	1.6	-	-	-	0.4	0.9	-	-	-	-	-	-	85	11	1.1	0.2	0.4	-
1,2-Dibromo-3-chloropropane	-	706	265	-	-	23	-	30	-	-	-	-	-	-	-	-	-	-	-	-
1,2,4-Trichlorobenzene	4.0	8.3	2.9	-	-	1.0	0.5	1.6	0.7	-	0.7	0.5	0.2	5.1	31	11	11	24	14	3.6
Hexachlorobutadiene	-	0.3	0.3	-	-	-	-	-	-	0.2	-	-	-	3.8	12	1.6	5.4	6.9	1.5	0.4
Naphthalene	1.9	3.5	2.9	-	63	1.6	-	3.3	1.9	4.0	2.0	-	-	3.1	2.7	1.7	2.3	1.5	1.9	2.0
1,2,3-Trichlorobenzene	-	3.3	-	-	-	-	-	-	-	-	-	-	-	-	6.2	5.8	1.7	5.4	10	2.3

[1] Values below LOD, as given in Table 3.3.2, are reported as □ [2] For locations, see Table 3.3.1.

principal component analysis, seemed inappropriate because of the limited number of statistical cases. Nonetheless, a correlation analysis was performed for the concentrations of the reported VOCs. While no significant correlation was found for any of the other VOCs, the BTEX compounds were found to correlate extremely well with each other, with correlation coefficients of between 0.77 and 0.98, and on average 0.89 better ($p<0.005$, $n=20$).

The fairly high concentrations found in this study do not come as a complete surprise: the general picture agrees with earlier observations, which, actually, triggered this work. The earlier studies showed that various VOCs were present in both marine organisms and in eel from the Scheldt estuary [11,13]. In Fig. 3.3.5 the concentrations of a number of priority VOCs in marine organisms from the Belgian coastal water are compared with the results of this study. The concentrations of the chlorinated hydrocarbons (CHCs) are seen to be generally significantly lower in marine fish than in eel from inland waters. This is the case even for lipid-rich tissues such as the liver. Literature data on CHCs in eel are very limited. An exception is the overview by Howard [10] which reports tetrachloroethene concentrations in American eel of 105 –250 ng/g that are at least an order of magnitude higher than in marine organisms. This is similar to what is observed here. Especially for this analyte, the observed median concentrations are a lot higher in eel than in marine fish. Tetrachloroethene has a limited bioconcentration capacity and accumulation occurs in the lipid-rich tissues of both man and animals [16]. The higher observed levels in eel are therefore more than likely the result of a higher exposure of freshwater organisms to this compound. The same also seems to apply to the other CHCs, although to a lesser extent. The difference is probably related to differences in uptake and metabolisation rates and the lower bioconcentration capacity of the other CHCs.

In contrast to the CHCs, median concentrations of BTEX in eel are more or less the same as those found in the liver of marine fish, with the exception of, perhaps, toluene. In contrast to CHCs, BTEX emissions are not solely related to industrial processes, i.e. local sources. BTEX were indeed found at all sampling locations and the variability of the data is somewhat less than for the other VOCs (Fig. 3.3.4). BTEX are common constituents of diesel oil and many petrochemical products, and are emitted in the exhaust gases of combustion engines [5,10,17]. This fits well with the observed correlation between the BTEX compounds and is in line with our earlier observations on VOCs in marine

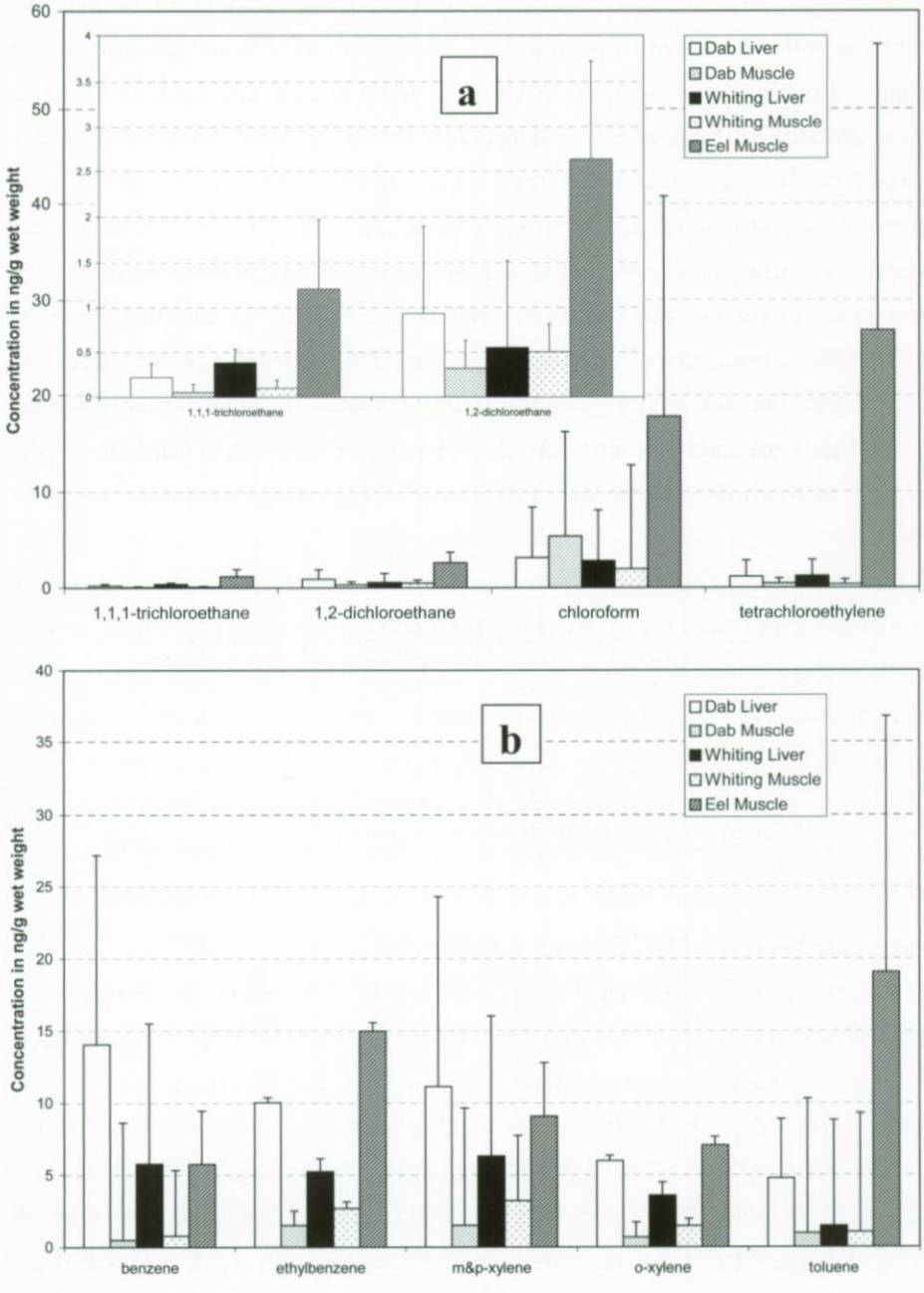

Figure 3.3.5: Comparison of the concentrations of selected (a) CHCs and (b) BTEX in tissues of marine species and freshwater eel.

organisms [11]. In that study, the observed correlation for these compounds was related to this common source and it was suggested that the principal source of BTEX in marine organisms is the use of fossil fuel. Dewulf *et al.* [3] observed higher levels of MAHs (monoaromatic hydrocarbons) than of CHCs in water and air samples from the same region and attributed this also to anthropogenic emissions from marine traffic in this coastal area. The same group also carried out an extended study of VOCs in the water column of the estuary of the Scheldt river and found similar results for BTEX in the water column [3]. These authors observed significant correlations between the various BTEX and a more uniform distribution of the concentrations throughout the estuary compared to CHCs. BTEX concentrations in this study were also of the same order of magnitude as in the marine environment, which was not the case for CHCs. These observations support the hypothesis that contamination by BTEX is of a rather diffuse nature which, in its turn, supports the conclusion that the use of fossil fuel in, e.g. traffic, is the major source of BTEX.

Table 3.3.4: Correlation matrix for BTEX compounds*.

	Benzene	Toluene	Ethylbenzene	m&p-Xylene	o-Xylene
Benzene	1.00	0.96	0.77	0.90	0.92
Toluene	0.96	1.00	0.77	0.92	0.95
Ethylbenzene	0.77	0.77	1.00	0.90	0.80
m&p-Xylene	0.90	0.92	0.90	1.00	0.98
o-Xylene	0.92	0.95	0.80	0.98	1.00

*Reported coefficients are significant at $p<0.05$ (n=20)

Spatial distribution of VOCs and eel as a biomonitor

The current database is too limited to allow an analysis of the spatial distribution for all VOCs included in this study. Such a comparison is justified only for the most prominent VOCs. That is, the comparison was limited to chloroform and tetrachloroethene, and the BTEX compounds. The latter are considered as a group based on the correlation discussed above (Table 3.3.4), and are represented by their sum. Fig. 3.3.6 gives an overview for the selected VOCs per sampling station and river basin. The patterns for eel collected at the same locations (Albertkanaal, Grensmaas, Kanaal Leuven-Dijle) are closely similar both with regard to the concentrations and their ratios. As regards the different river basins, the VOC concentrations in eel from highly industrialized and populated regions (Dender, Dijle-Zenne and Nete basins) are higher. This is especially

true for BTEX. The high concentrations observed at the Groot-Zuunbekken station can possibly be explained by the fact that this is a pond in a densely populated and industrialized area, which is in the vicinity of a tributary of the Zenne river, the Zuunbeek, which is biologically dead. Probably, water form the brook entering the pond explains the observed results. Since there is little exchange with surrounding water masses, VOCs are lost probably only as a result of evaporation. As this is a dynamic process, it would indicate a constant high level of input into that water body. In marked contrast, eels from rural locations, such as the A at Poppel, show a significantly lower concentration.

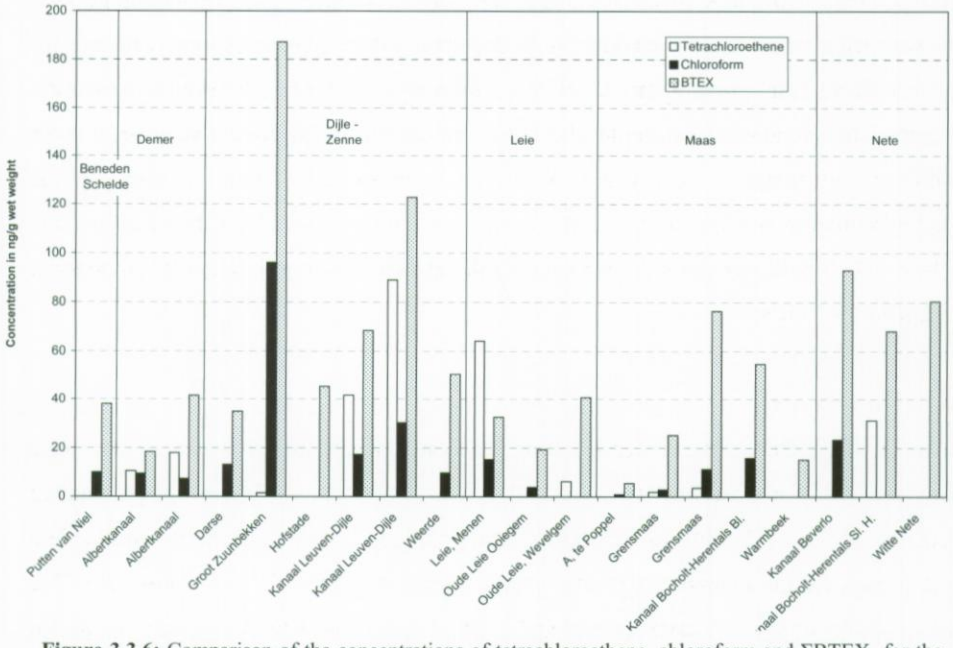

Figure 3.3.6: Comparison of the concentrations of tetrachloroethene, chloroform and ΣBTEX for the various sampling stations.

Recent data for the concentrations of the same VOCs as were studied here in the water columns of Flemish rivers show that these are generally below the LODs of the analytical techniques used, i.e. 0.05–2 µg/l. That is, they are below the current water-quality criteria of the Flemish government, which are set at a median value of 2 µg/l for total VOCs and 1 µg/l for each individual VOC [18]. Not surprisingly, the VOCs that were detected in the water columns, are the same as the most prominent ones in this study and the highest concentrations are also found in the Dijle-Zenne basin. Taking into account that the bioconcentration factor (BCF), *viz.* the ratio of the concentrations of an analyte in the

organism and the water, is between 1 and 90 for most VOCs [5,19,20], the concentrations found in eel are not surprising. For instance, if the LODs of the BTEX compounds in water are taken as the actual concentrations (0.2–0.4 µg/l), concentrations of 20–40 ng/g would be expected in eel if an estimated log BCF of 2 is used [5]. As can be observed from Fig. 3.3.4, median values of approx. 10 ng/g were found for the various sampling stations in our study. This leads to the conclusion that concentrations in eel indeed reflect the concentrations in the water column. Moreover, the – admittedly, limited – information presented above shows that eel samples from the same location have similar patterns and VOC concentrations. There is evidence to assume that once contaminants are stored in the lipid, they will not be metabolised and thus become resident. Also because eel do not spawn during their stay in inland waters, the observed concentrations are valuable for time-trend analysis, and, because eel is essentially sedentary and normally does not migrate, concentration data should allow the comparison of different river systems. An additional advantage is that yellow eel are known to reflect rapid changes in the concentrations of organic contaminants in the surrounding water [12]. In summary, the yellow eel *Anguilla anguilla L.* can be considered as a potential biomonitor or sentinel organism for VOCs.

Hazard assessment

In a previous study, the observed concentrations in the marine environment were compared with proposed safety levels. The approach used was based on quantitative structure–activity relationships (QSARs), extrapolation of toxicity data and equilibrium partitioning for the assessment of the effects of narcotic industrial pollutants [21]. The extrapolation of toxicity data generated by QSARs was used to derive safe levels for water, sediment and biota. The model allows the calculation of internal toxic concentrations (ITCs) in fish tissue, which is useful for the interpretation of biomonitoring data. The safety level was arbitrarily set at 95%. This implies that a threshold concentration, the hazardous concentration HC5, is calculated which is unlikely to cause harm to more than 5% of the aquatic community. However, the usefulness of the model hinges on the applicability of the equilibrium-partitioning theory and its relation with octanol–water partitioning. The latter seemed certainly the case for marine species and there are no indications why it should not be true here. The observed levels were therefore tentatively compared with HC5 values calculated during the previous study.

Table 3.3.5: Comparison between observed VOC concentrations (ng/g) and HC5 values (ng/g) calculated according to Van Leeuwen *et al.*[21].

Location	Concentrations (ng/g)							
	benzene	toluene	p-xylene	o-xylene	chloroform	tetrachloroethene	1,2-dichloroethane	1,1,1-trichloroethane
HC5:	**5200**	**5900**	**6400**	**6500**	**8100**	**9700**	**6700**	**8800**
Leie, Menen (1)	3	11	8	6	15	64	-	2
Albertkanaal, Langerlo (2)	2	5	3	2	9	11	2	-
Kanaal van Leuven naar de Dijle, Tildonk (3)	7	33	9	7	17	42	-	-
Groot Zuunbekken, St.-Pieters-Leeuw (4)	19	73	35	40	96	2	-	-
Kanaal van Leuven naar de Dijle, Tildonk (5)	10	47	18	12	30	89	-	-
Grensmaas, Molensteen (6)	3	7	4	3	3	2	-	-
Oude Leie Ooigem (7)	3	7	3	2	4	-	-	-
Witte Nete, Dessel (8)	11	41	9	9	-	-	-	-
Pond at Rijksdomein, Hofstade (9)	5	14	8	4	-	-	3	-
Grensmaas, Molensteen (10)	7	20	13	7	11	4	3	-
Zandwinningsput, Weerde (11)	5	13	8	5	10	-	4	-
Albertkanaal, Langerlo (12)	4	12	7	5	7	18	3	1
A, te Poppel (13)	1	2	1	1	1	-	-	-
Kanaal Bocholt-Herentals, Blekerheide (14)	9	22	11	8	-	31	5	2
Oude Leie, Wevelgem (15)	6	11	7	5	-	6	-	-
Putten van Niel, Niel (16)	4	11	6	4	10	-	1	-
Kanaal Bocholt-Herentals, Sluis Herentals (17)	4	11	10	6	16	-	2	1
Warmbeek, Achel (18)	2	4	2	2	-	-	-	-
Darse, Vilvoorde (19)	4	9	6	4	13	-	2	-
Kanaal Beverlo, Leopoldsburg (20)	7	30	15	12	23	-	-	-

Values below LOD (see Table 3.3.2).

Table 3.3.5 shows the HC5 values for some selected VOCs and their concentrations measured at the various sampling stations. The results show that in no case the HC5 is exceeded. Moreover, the experimentally determined concentrations are several orders of magnitude lower than the HC5. One may therefore assume that, in all likelihood, this is also true for those VOCs for which no HC5 data are available. On the other hand, one should note that the hazard assessment does not take into account synergistic and, thus, more damaging effects. To quote an example, the eel from Groot Zuunbekken, with the highest concentrations of VOCs, did have an abnormally low lipid content, *viz.* 9% compared to an average of 25%. Nevertheless, more definite statements regarding long-term effects cannot, as yet, be made because the dataset is far too small and the calculation of the HC5 is only one approach amongst several and needs to be further evaluated. That is, additional research, especially with regard to the long-term consequences of small doses of VOCs is required and the use of eel as sentinel organisms for VOCs should be studied in more detail.

3.3.4 Conclusions

A number of important VOCs are present in eel from Flemish inland waters. The most abundant VOCs are BTEX and the chlorinated VOCs, chloroform and tetrachloroethene. In general, the concentrations of the chlorinated VOCs are higher in eel than in the lipid tissue of marine fish. However, this is not true for the BTEX, for which the levels are comparable to marine fish; this can be explained by the much more diffuse nature of the sources for BTEX.

The present exercise indicates that the VOC concentrations in eel reflect the actual concentrations in their environment. Also, if the BCFs and the concentrations in the water column are taken into account, the observed levels are well in line with expectations. In other words, eel is a potential biomonitor or sentinel organism for VOCs and further study is justified. This should include extended sampling at given locations and a more in-depth study of the behaviour of VOCs in the organism. For the rest, a follow-up study should be sufficiently wide-ranging to allow evaluation of the long-term consequences of small doses of VOCs and their synergistic effects.

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4 Compliance monitoring

4.1 PCBs in cod (*Gadus morhua*), flounder (*Platichthys flesus*), blue mussel (*Mytilus edulis*) and brown shrimp (*Crangon crangon*) from the Belgian continental shelf: relation to biological parameters and trend analysis[§§]

Abstract

PCB levels in cod, flounder, mussel and shrimp, covering a ten-year period, were assessed for temporal trends and their relation to biological parameters. A significant relation was found between the PCB levels on a wet weight basis and the total lipid content. Normalising on the total lipid content reduced the differences in PCB levels between the organisms and between different tissues within the organisms. A general downward trend was observed for the PCB levels on the Belgian continental shelf.

[§§] From *Chemosphere*, 37 (1998) 2199-2210.

4.1.1 Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants, which have caused world-wide concern since the discovery of their presence in the environment by Jensen [1]. Their widespread occurrence in the marine environment and their toxic potential resulted in a number of international monitoring programmes such as the Co-ordinated Monitoring Programme of the International Council for the Exploration of the Seas (ICES) and the Joint Monitoring Programme (JMP) of the Oslo and Paris Commissions (OSPARCOM) [2]. These programmes aimed to assess the levels of PCB contamination in the marine environment with an emphasis on human consumption and the overall quality of the marine ecosystem, and to investigate possible trends in PCB levels.

The Belgian Fisheries Research Station has been measuring PCBs in marine samples since 1978. The data presented here cover an eleven-year period (1983-1993) and form a solid basis to investigate time trends. Trends in the PCB concentrations in cod, flounder, mussel and shrimp on the Belgian continental shelf in relation to biological parameters such as fat content, age, weight, length and sex are assessed.

4.1.2 Materials and Methods

Materials

All materials used for this work were of research grade quality. Standard solutions were prepared on a weight basis from pure compounds (> 99% pure) or certified reference standards.

Sampling

Cod (*Gadus morhua*), flounder (*Platichthys flesus*) and shrimp (*Crangon crangon*) were collected by the institute, using beam trawling, on the Belgian continental shelf from 1983 to 1993. Twenty-five individuals per fish species were sampled 2-3 months prior to spawning and divided in five length classes between 214 and 905 mm for cod and 200 and 450 mm for flounder. Muscle tissue was analysed individually but livers were pooled per length class. Shrimp sample sizes comprised 100 individuals. Cooked tail muscle was isolated and divided in five subsamples. Mussels (*Mytilus edulis*) were harvested on three jetties along the Belgian coast and sorted per length class of 20-30 mm, 30-40 mm, 40-50 mm and > 50 mm. Total sample sizes were between 150 and 617 individuals. The mussels

were left in settled seawater at room temperature for 24 hours. Subsequently the soft body was isolated for analysis. All samples were stored at -28 °C prior to analysis.

Chemical analysis

Extraction was based on total lipid extraction according to the method of Bligh and Dyer [3]. The extracted lipids firstly used for the determination of the fat content were redissolved in hexane, and the resulting solution was subsequently cleaned on a Florisil column [4,5]. Analyses were performed on a Carlo Erba 4160 gas chromatograph equipped with an electron capture detector and a 25 m SE-54 column (before 1990) or (from 1990 onwards) a 60 m DB-17 and a 60 m DB-5 column (internal diameter 0.25 mm, film thickness 0.25 μm). Prior to 1989, the PCB concentrations were calculated on the basis of comparison with eight PCB peaks of Aroclor 1260 [4]. These eight peaks corresponded with IUPAC nos. 101, 136, 147, 153, 138, 128, 180 and 170 [6]. Concentrations of individual congeners, viz. IUPAC nos. 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180 [6], are determined since 1989 [5]. Prior to 1990, quality assurance consisted of the analysis of procedural blanks, reproducibility and repeatability tests, injection of standard solutions as unknowns, and analysis of samples with known concentrations. Since 1990, the analysis of a certified reference material (BCR CRM 349) has been added as a standard procedure.

Conversion of data calculated with the Aroclor standard.

Due to the lack of individual PCB congener concentrations, before 1989, all statistical analyses had to be performed on the total PCB concentrations. Since 1989 individual congener concentrations are calculated and summed to express the total PCB concentration (ΣPCB). However, the resulting sum is not equal to the concentration calculated on the basis of Aroclor 1260 (Aroclor concentration). A conversion or recalculation method was therefore developed. The conversion is based on the fact that the ratio between the total PCB concentrations calculated with both methods should remain constant if the PCB patterns are identical and the ratios of the individual peaks to the total peak pattern are constant. A conversion factor (CF) can then be calculated, which is given in Equation 1.

$$(1) \qquad CF = \frac{[Aroclor]}{\sum PCB}$$

with [Aroclor] = concentration based on Aroclor 1260 and ΣPCB = summed concentration of individual PCBs

Consequently the older Aroclor concentration can now be recalculated to give ΣPCB values. All ΣPCB referred to in this paper are either the sums of individual congeners or the concentrations recalculated as described above.

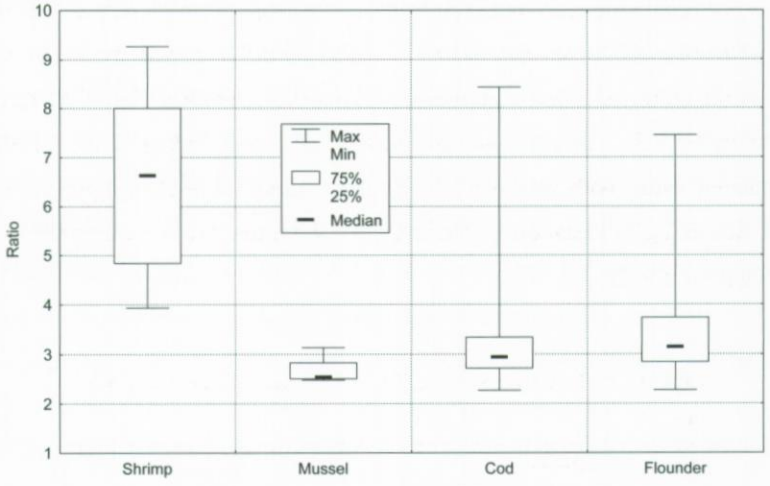

Figure 4.1.1: Ratio between ΣPCB and CB 153 for shrimp, mussel, cod and flounder in the period 1990-1993.

Statistical analysis

All statistical analyses were performed on ΣPCB and the level of significance was set at 95%. Correlations between fat content, length of the animal and PCBs were analysed by linear regression. The non-parametric Mann-Withney test was used to investigate the relation between sex and PCB concentrations, and the non-parametric Kruskall-Wallis ANOVA test combined with Dunns' post test was performed to compare the PCB contents of liver and muscle tissues in and among species and to study the influence of the age of the animals on the PCB content. Time-trend analysis of the PCB concentrations (median values per year) in cod, flounder and mussel were studied according to the method of Nicholson *et al.* [7]. PCB trends in shrimp were analysed by linear regression.

4.1.3 Results

Recalculation of the Aroclor concentrations

Converting the Aroclor concentrations to ΣPCB by using Equation 1 requires that the PCB pattern of a given species/tissue is identical and that the ratios of the concentration of individual peaks to the total peak pattern are constant. The ratios between ΣPCB and CB 153 were calculated for the data obtained since 1990 and are presented by box and whisker plots in Figure 4.1.1. The results show a narrow box for cod, flounder and blue mussel and prove that the ratio remained constant; they also allow to suggest that the PCB patterns are similar. However, the shrimp data show a different pattern, which may find its origin in the rather small data set available. The PCB concentrations in the samples of cod and flounder, taken in 1991, and of mussel and shrimp, taken in 1991-1992, were then calculated using both methods of calculation and for each sample the CF was calculated according to Equation 1 (Table 4.1.1). Next, the Aroclor concentrations were re-calculated into ΣPCB.

Table 4.1.1: Conversion factors (CF) for the recalculation of 'Aroclor' data.

Species	CF
Cod	4.20 ± 0.04 (n=25)
Flounder	3.6 ± 0.1 (n=25)
Mussel	3.4 ± 0.1 (n=12)
Shrimp	3.1 ± 0.1 (n=10)

In principle, the CF value of 3.1 cannot be used to recalculate the Aroclor concentrations of shrimp, since the experimental results do not provide the required proof. However, the standard deviation of the CF is rather small; it is, moreover, similar to that of the other species. Moreover, a similarity between PCB patterns in invertebrates has been reported in the literature [8,9] and is indeed found for mussel (cf. above). We therefore assumed that the PCB patterns in the same species of invertebrate from the same location will be essentially the same and used the CF-based procedure also to recalculate the older data for shrimp.

Relation between PCB concentrations and total lipid content.

The results of the correlation analysis between the total lipid content and log (∑PCB) for the different species and tissues are given in Table 4.1.2. A significant correlation ($p<0.05$) was found for the log (∑PCB) expressed on a wet weight basis and the total lipid content, despite the large variability of the data (Figure 4.1.2). No significant correlation was found when the concentrations were normalised on the total lipid content (Table 4.1.2).

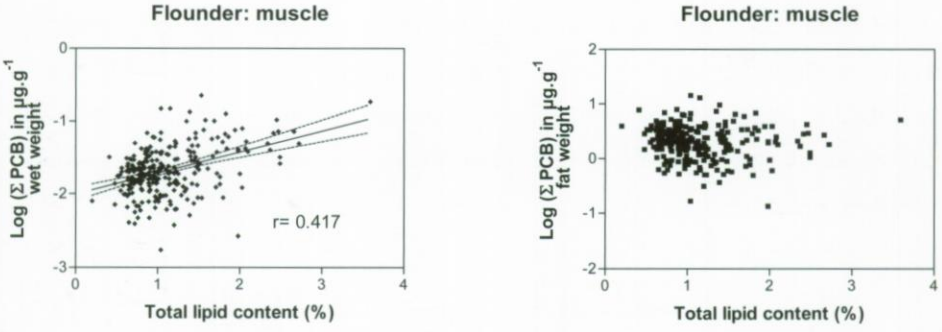

Figure 4.1.2: Relationship between total lipid content and log (ΣPCB) expressed on wet and fat weight basis (r, correlation coefficient; dotted line, 95% confidence interval of the mean).

The effect of lipid normalisation of the PCB data is illustrated in Figure 4.1.3 for all species and tissues examined. The results of a Kruskal-Wallis ANOVA analysis of the data indicate significant ($p<0.05$) inter-tissue and inter-species differences. However, narrowing this down with Dunn's post test revealed that the differences between cod liver, flounder liver, flounder muscle and blue mussel (soft body tissue) were not significant. Obviously, normalisation on the total lipid content reduces the differences in PCB levels between the organisms and between different tissues within the organisms, that is, the results illustrate the importance of lipids as a normalising factor. PCB concentrations are therefore only considered on a fat weight basis in the remainder of this paper.

Table 4.1.2: Results of correlation analysis between total lipid content and log (ΣPCB) on wet and fat weight basis for the different species and tissues.

Parameter	Total lipid content (%) vs. log (ΣPCB) on wet weight basis		Total lipid content (%) vs. log (ΣPCB) on fat weight basis	
	r	p	r	p
Cod muscle	0.25	<0.05	-	0.7255
Cod liver	-	-	-	-
Flounder muscle	0.42	<0.05	-	0.1392
Flounder liver	-	-	-	-
Blue mussel	0.50	<0.05	-	0.7526
Brown shrimp	0.51	<0.05	-	0.7685

p = p value, r = correlation coefficient

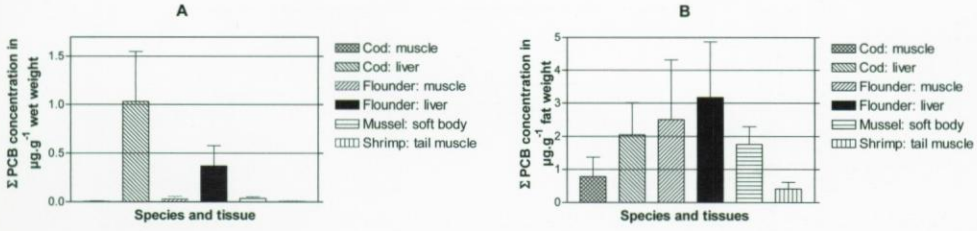

Figure 4.1.3: PCB concentrations for cod, flounder, mussel and shrimp, (A) not normalised and (B) normalised on total lipid content.

Relations between ∑PCB and length and sex

The trend analysis of Nicholson *et al.* [7] dictates a different approach when a length effect has been established. As regards both fish species, no demonstrable size effects were found except in cod liver (Figure 4.1.4). The ∑PCB concentrations were in addition to body size also related to sex, but no significant relations were found.

As a result of the sampling procedure, no individual size data were available for the invertebrates. For mussel, however, samples were divided into five length classes and the ∑PCB concentrations were compared. The results are shown in Figure 4.1.5. The length class has, apparently, no effect on the ∑PCB concentrations, which was confirmed with a Kruskal-Wallis ANOVA test.

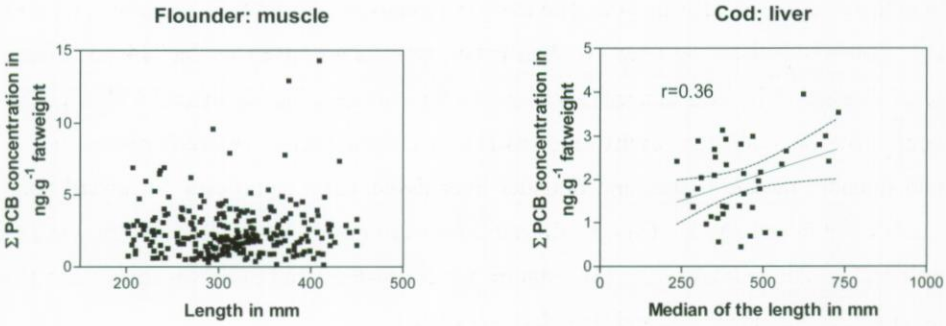

Figure 4.1.4: Relationship between length and log (∑PCB) expressed on fat weight basis for flounder muscle tissue (left) and cod liver (right) (line illustrates calculated significant trends; r, correlation coefficient; dotted line, 95% confidence interval of the mean).

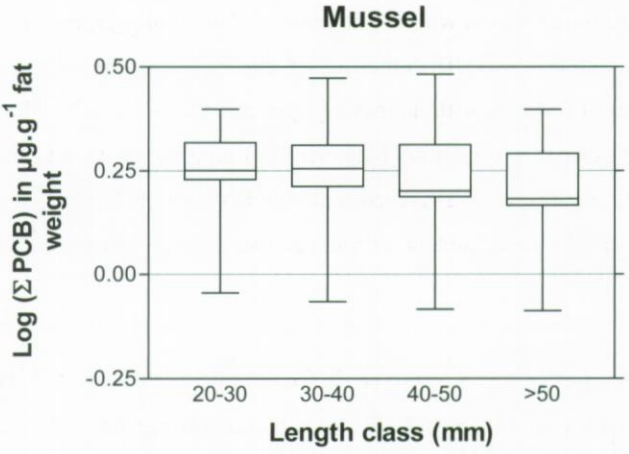

Figure 4.1.5: Relationship between length class and log (∑PCB) expressed on a fat weight basis for blue mussel (box, median and 25 and 75 percentiles; whiskers, minimum and maximum).

Temporal-trend analysis

The observed absence of relations between the PCB content and the animals' length or sex allowed the analysis of temporal trends in cod muscle, flounder muscle and flounder liver tissues and in blue mussel without statistical modifications, but not for cod liver with which a length effect was found, nor for brown shrimp for which the length effect was not studied. As regards the cod liver data, they were subdivided at the median into a 'small' and a 'large' group and both were analysed independently [7]. Temporal trends in brown shrimp data were analysed by linear regression. The data were log transformed in order to

approach the normal distribution. The temporal trends are illustrated in Figure 4.1.6 and the lipid normalised mean and median concentrations are given in Table 4.1.3. Long-term changes in the PCB concentrations were only considered as significant within a 95% confidence interval. The results revealed (1) significant year-to-year differences in cod and flounder muscle tissues and flounder liver tissue, (2) a significant downward non-linear trend in cod muscle, (3) a significant downward linear trend in flounder muscle, (4) no trend in blue mussel tissue, (5) a significant downward trend in brown shrimp and (6) no significant trends in cod and flounder liver tissues.

4.1.4 Discussion

For the four species studied the PCB concentrations expressed on a wet weight basis show a significant correlation with the fat content. This finding agrees well with previous observations. Schaefer *et al.* [10] demonstrated that PCB concentrations per wet weight in different tissues of cod rose with increasing lipid content as did Schneider [11]. Goerke *et al.* [12] found positive correlations between PCB concentrations and the fat content of various marine organisms. Positive correlations between PCB concentrations on a dry weight basis and the lipid content of various marine organisms were also reported by Delbeke *et al.* [13].

Moreover, inter-species and tissue-type differences decreased when PCB concentrations were normalised for the fat content. The correlation between fat content and log ($\sum$PCB) illustrates the need for a normalisation of the PCB concentrations on a fat basis, especially when a time-trend assessment is attempted. The explanation probably is that the natural variations in the lipid content of an organism or organ, due to e.g. spawning or lack of food, may influence the variability of contaminant data when these data are expressed on a fresh (wet) weight basis. Delbeke *et al.* [13] observed a similar reduction of the inter-species variability of PCB isomer concentrations after normalisation of the data on 'total neutral lipids', as determined by Iatroscan analysis. Using this selected class of lipids for normalisation proved superior to using the total lipid content (gravimetrically determined). The authors concluded that this kind of normalisation may provide a basis for extrapolation of PCB pollution data among species. However, the inter-tissue and inter-species variability of our contaminant data is on the same order of magnitude as that

observed by Delbeke *et al.* [13]. Consequently, there may be some doubt whether speciation of the lipids would give an improvement in this case.

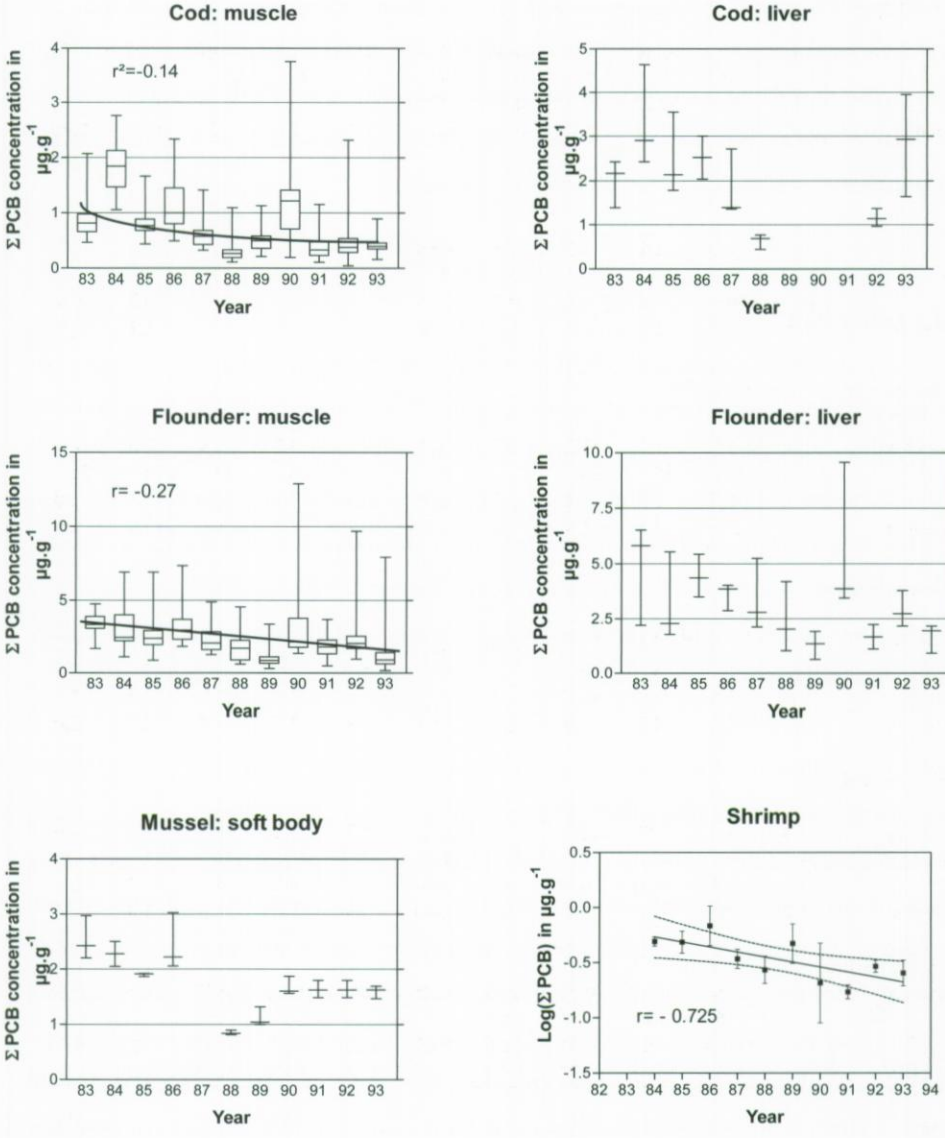

Figure 4.1.6: ΣPCB concentrations in µg/g fat weight for cod, flounder and mussel (lines illustrate calculated significant trends; r , correlation coefficient; boxes, median and 25 and 75 percentiles; whiskers, minimum and maximum) and linear trend for the log(ΣPCB) concentration in shrimp (r, correlation coefficient; dotted line, 95% of the mean).

Table 4.1.3: Mean and median concentration (µg/g fat weight), standard deviation (s) and number of samples (n) for the different species in the period 1983-1992.

	83	84	85	86	87	88	89	90	91	92	93
Cod muscle tissue											
Average	0.87	1.8	0.84	1.2	0.64	0.33	2.1	1.3	0.44	0.57	0.43
n	25	18	25	25	25	25	25	25	25	25	25
s	0.34	0.5	0.29	0.5	0.28	0.23	0.9	0.9	0.31	0.54	0.15
Median	0.81	1.9	0.77	1.0	0.58	0.26	2.1	1.2	0.33	0.38	0.40
Cod liver											
Average	2.0	3.2	2.5	2.5	1.7	0.64				1.2	2.7
n	5	5	5	5	5	5				5	5
s	0.5	0.9	0.7	0.4	0.6	0.14				0.2	0.9
Median	2.2	2.9	2.1	2.5	1.4	0.69				1.1	2.9
Flounder muscle tissue											
Average	3.4	3.1	3.1	3.2	2.5	1.9	3.8	3.3	1.8	2.5	1.7
n	25	20	25	25	25	25	25	25	20	25	25
s	0.8	1.7	1.8	1.2	1.1	1.2	2.4	2.7	0.7	1.8	2.0
Median	3.3	2.4	2.4	2.9	2.0	1.7	3.1	2.1	1.8	2.0	0.9
Flounder liver											
Average	4.9	3.3	4.4	3.6	3.3	2.5	4.6	4.9	1.7	2.8	1.8
n	5	5	5	5	5	5	5	5	4	5	4
s	1.8	1.6	0.8	0.45	1.2	1.3	1.9	2.6	0.5	0.6	0.6
Median	5.8	2.3	4.4	3.9	2.8	2.0	4.8	3.9	1.7	2.7	2.0
Blue mussel											
Average	2.5	2.3	1.90	2.3		0.85	3.7	1.7	1.6	1.3	1.6
n	4	4	4	4		4	4	4	4	4	4
s	0.3	0.2	0.03	0.4		0.04	0.5	0.1	0.2	0.2	0.1
Median	2.4	2.3	1.91	2.2		0.84	3.6	1.6	1.6	1.3	1.6
Brown shrimp											
Average		0.50	0.49	0.71	0.35	0.28	0.49	0.21	0.17	0.29	0.26
n		13	5	5	5	5	5	2	5	5	5
s		0.08	0.09	0.21	0.06	0.07	0.18	0.02	0.02	0.03	0.05
Median		0.49	0.47	0.72	0.33	0.26	0.47	0.21	0.18	0.29	0.28

Length and sex had no noticeable effects on the PCB concentrations expressed on a fat weight basis, with one exception: PCB concentrations in cod liver significantly increased with length. An influence of the length of cod on the PCB content in the liver was previously reported by de Boer [14], who demonstrated a significant concentration

difference between individuals of different sizes (53-54 cm and 85-91 cm). Similarly, Kruse and Krüger [15] measured higher DDT levels in liver of Baltic cod of larger size, but they did not notice similar trends for hexachlorobenzene (HCB), α-hexachlorocyclohexane (α-HCH) or dieldrin. Bioaccumulation of contaminants such as PCBs in biota is the result of a combination of uptake (directly from the water, ingestion of contaminated particles and food) and elimination (metabolisation, excretion, growth dilution, spawning). The relative importance of each process will, of course, depend on the species considered and its stage of life. An explanation for the size-dependent contaminant level in cod liver may be found in the regime of larger cod. Larger cod mainly feeds on fish, which is more contaminated than invertebrates that are preferentually consumed by smaller fish [16]. The major route of PCB uptake in larger cod, food, will therefore cause biomagnification. The bioaccumulation in muscle tissue is, however, not size-dependent; this may be related to the fact that lipid deposition with cod is mainly in the liver. The food consumption pattern of flounder, mussel and shrimp does not change during their life cycle [17]. For those species, no significant biomagnification was found; obviously, the uptake of PCBs is compensated by elimination processes.

Significant downward trends were observed in muscle tissue of cod and flounder, and in shrimp, but not in mussel and the liver of both fish species. From among these species, flounder, blue mussel and brown shrimp are excellent indicator organisms which clearly reflect the quality status of their habitats because of no or restricted migratory activities. Cod has a more enhanced migratory behaviour and does not necessarily reflect the condition of the area of capture. Nevertheless, cod is considered to be a suitable biomonitor for spatial and temporal trend monitoring. Migration appears to be sufficiently confined and allows observing differences between regions that are some hundred kilometres apart [18]. The observed temporal trends in this study are on the same tenor as others recently reported. In the 1993 North Sea Quality Status Report [2], decreasing PCB contents were cited for several species and various locations were cited and recent observations revealed decreasing concentrations of lower-chlorinated PCBs in yellow eel (*Anguilla anguilla*) from inland waters in the Netherlands [19]. PCB concentrations in cod (*Gadus morhua*) from the North Sea have been shown to have decreased significantly, although higher chlorinated congeners remained at an essentially constant level [19]. Constant PCB contents were reported by Stronkhorst [20] for *Mytilus edulis* and by Solé *et al.* [21] for *Mytilus galloprovincialis* from the western Mediterranean. The

observed trends may well indicate that PCB concentrations have reached their maximum values and that the compulsory remedial actions implemented by national and international organisations to improve the quality of the marine environment gradually become successful. However, although PCB concentrations are shown to decrease regionally, a global decline is not expected in the next few years, because of on-going inputs into the environment caused by, *e.g.*, leakages from landfills and emissions from incinerators [22]. This was emphasised recently during the latest assessment of the OSPAR coordinated environmental programme at the MON 2004 meeting [23]. Moreover, it has been stated that the quantities of PCBs still in use, still exceed the amount that has been released into the environment to date [22]. A nice illustration is the recent the 'dioxin' crisis in Belgium where poultry were severely contaminated by PCBs through an illegal addition of a PCB containing oil to oils used for the preparation of their feed [24].

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4.2. Polychlorinated biphenyls in marine sediments from the southern North Sea and Scheldt estuary: a ten-year study of concentrations, patterns and trends[***]

Summary

The paper reports the concentrations and patterns of CBs in sediments of the Belgian part of the southern North Sea and the Scheldt estuary for the period 1991-2001. The long-term analytical performance was well within the quality assurance boundaries set at the outset of the study and is consistent with the state of the art for this type of analysis. The CB concentrations (given as the median of the sum of IUPAC Nos 28, 52, 101, 118, 138, 153 and 180) vary between 0.1 µg/kg and 50 µg/kg in the total sediment and it could be demonstrated that CB patterns in the fine fraction of the sediment were closely similar throughout the investigated area. Isolation of the fine fraction (<63 µm) by sieving can be regarded as a physical normalisation to reduce the differences in sediment granulometric composition. It allows for a better understanding of CB distribution and patterns and improves the trend analysis. A significant downward trend could not be found at any of the stations, which suggests that CB levels have not been changing in the area of interest in the past decade.

[***] Submitted to *J. Environ. Monit.*, 2004.

4.2.1 Introduction

Polychlorinated biphenyls or PCBs (individual congeners, CBs) have been a major cause for concern since their discovery in the environment by Jensen [1]. Large amounts of technical mixtures of CBs were manufactured by companies in the US, Japan and several European countries between 1930 and 1983, when their production was discontinued [2]. During this period but, also, more recently, large quantities of CBs reached the environment through, *e.g.*, large-scale disposal, leakage, evaporation and accidents [3]. The widespread distribution of these contaminants in the marine environment and their high persistence rapidly raised questions about the hazards posed to marine life and the ecosystem as a whole. This was recognised early on by international marine organisations such as the Oslo and Paris Commissions (OSPARCOM). As a result, CBs became routinely monitored determinants in the Joint Monitoring Programme of OSPARCOM [4] and in its follow up, the Joint Assessment and Monitoring Programme (JAMP). Notably, in order to identify impacts of concern, human pressures on the North Sea environment were ranked by OSPAR into four priority classes, with the identification of organic microcontaminants belonging to the first priority class [5]. Recommendations call for stronger efforts in the implementation of the OSPAR Strategy on Hazardous Substances, which implies the prevention of pollution of the maritime area by continuously reducing discharges, emissions and loss of hazardous substances, with the ultimate aim of achieving concentrations in the marine environment close to background values for naturally occurring substances and close to zero for xenobiotics.

Since the early eighties, CBs are routinely monitored in a variety of marine samples, specifically, organisms and sediments. Analysis is almost invariably done by capillary gas chromatography (GC) with selective, *i.e.* electron-capture (ECD) or mass-spectrometric (MS) detectors. In the past two decades, a reduction of contaminant concentrations in the marine system similar to that achieved for emissions, discharges and losses has not been observed, certainly not for sediments and biota [5]. The general absence of decreasing trends might originate from the fact that most time series are still too short to reveal reliable information on trends, from a high natural variability of contaminant levels, from an insufficient sampling frequency and/or from too rigorous statistical requirements.

For sediments, the influence of the natural variability in sediment composition, *i.e.* grain-size distribution, organic matter and mineralogy, has to be taken into account when

performing a trend analysis. Organic microcontaminants such as CBs show a much higher affinity to fine particulate matter compared to sandy material, because constituents such as organic matter and clay minerals contribute to the specific surface area of this fine material. In areas with varying grain-size distributions, the spatial distribution of contaminant concentrations will, therefore, be closely related to the distribution of fine-grained sediments, and effects of other sources of contaminants, such as anthropogenic sources, will be at least partly obscured by the effects of the grain-size differences [6]. Fine material, inorganic as well as organic, and associated contaminants are preferentially deposited in areas of low hydrodynamic energy, while in areas of higher energy, fine particulate matter is mixed with sandy sediment particles, which are generally not able to bind contaminants, as organic carbon (OC) is absent. This dilution effect will cause lower and more variable contaminant concentrations in the resulting sediment. It is, therefore, essential to correct for the effects of grain size.

Taking the grain size into account is especially important for the area under investigation. The southern part of the North Sea along the Belgian coast is a highly dynamic system of shallow, elongated sandbanks. The sediments consist of well-mixed fine- to medium-grain sands. Intensive sediment movements and associated sediment transport occur frequently, owing to wind-induced currents, tidal movement and/or wave action [5]. Sea swell is an especially effective agent for resuspension. These events lead to changes in seabed topography and may also result in resuspension of contaminant-containing settled particulate matter, its transportation, and deposition elsewhere. Due to the nature of the material and the quite different water flows, the influence of weather conditions, the transport and sedimentation of suspended particulate matter and the erosion of fine sediments are difficult to distinguish and to monitor.

The results evaluated in this paper comprise the Belgian contribution to OSPAR-JAMP for the period 1991-2001. The investigated area is the Belgian part of the southern North Sea and the Scheldt estuary. The paper reports the concentrations and patterns of CBs, discusses normalisation, and gives a preliminary trend assessment for CBs in sediments along the Belgian coast for the study period.

4.2.2 Materials and methods

Sampling

Samples were collected on board the RV Belgica at the various stations given in Figure 4.2.1. The geographical position of each station was carefully checked during all sampling campaigns. Sampling was performed according to the principles and guidelines of OSPARCOM [7]. At each location, the top 10-15 cm of the sediment was collected with a Van Veen grab sampler. The area under investigation is considered to be a transportational area, i.e. an area without a net sedimentation or erosion, where the upper 15-40 cm reflects the latest quality status of the mud. Subsequent results can be used for time trend monitoring [7b]. Immediately after sampling, the samples were stored at –28°C on board ship.

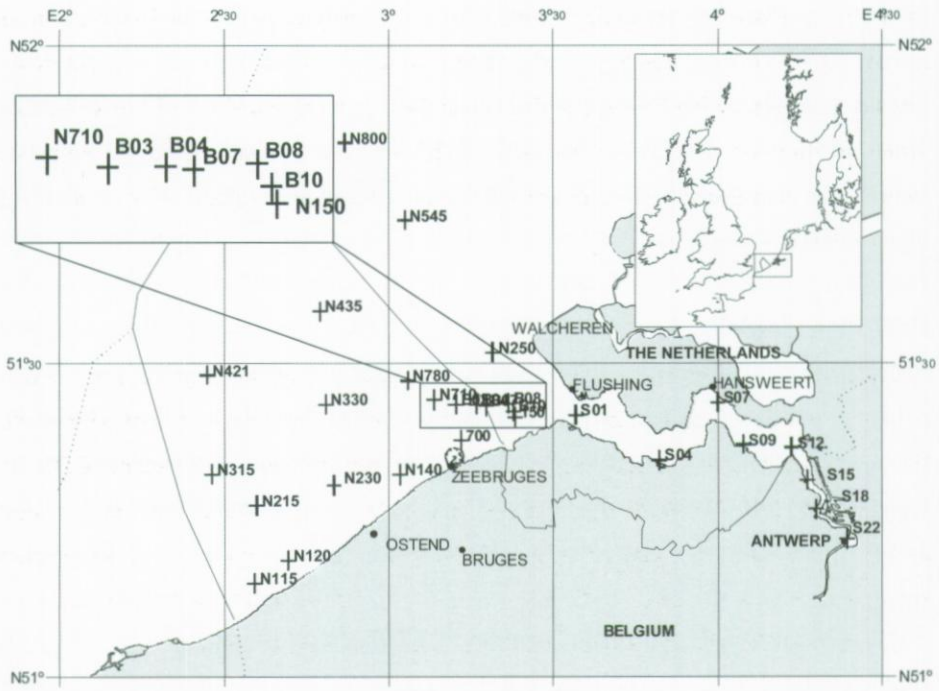

Figure 4.2.1: Geographical position of the sampling locations.

Materials

All reagents used for the analysis were of analytical quality. Hexane, iso-octane, acetone, isopropanol and diethyl ether were purchased from Promochem (Wesel, Germany).

Sodium sulphite, sodium sulphate, tetrabutylammonium sulphate, aluminium oxide and silica were purchased from Merck (Darmstadt, Germany).

Gases were also of analytical quality (alphagas 2; Air liquide, Liège, Belgium).

Sample preparation and extraction

Sediment samples were wet-sieved on a 63 µm sieve and lyophilised prior to analysis. Between 4 and 20 g of dry sediment were extracted with hexane/acetone (3/1) for 6 h in a hot Soxhlet apparatus [8]. Sodium sulphite in combination with tetrabutylammonium sulphate and isopropanol was added to remove most of the inorganic sulphur. Subsequently, the extract was concentrated to a volume of 1 ml in a rotary evaporator and under a stream of nitrogen, and brought onto a glass column containing an Al_2O_3/Na_2SO_3 mixture to remove any remaining sulphur [9]. The analytes were eluted with 25 ml of hexane and the extract was again reduced to a volume of 1 ml. This was brought onto a glass column containing 5% deactivated silica; the CBs were eluted with 17 ml of hexane. After addition of the internal standard, 1,2,3,4-tetrachloronaphthalene in iso-octane (which acts as a keeper), the mixture was again reduced to a volume of 1 ml and 1 µl injected on the GC column.

GC-ECD analysis

The analyses were performed on a Carlo Erba (Milan, Italy) 8000 gas chromatograph equipped with a 60 m x 0.25 mm Rtx®-5 MS capillary column (Restek) with a film thickness of 0.25 µm, a splitless injector and a ^{63}Ni electron-capture detector (ECD). The temperature of the injector was 235°C and that of the detector 310°C. Helium was used as the carrier gas and argon containing 5% methane as make-up gas. The temperature programme was as follows: 90°C during 2 min, from 90 to 150°C at 15°C/min, then at 3°C/min to 220°C, and at 1°C/min to 275°C, with a final hold of 10 min.

The GC system was calibrated by using a series of standard solutions with CB concentration ranging from 0.70 to 70 ng/ml. Standard solutions were prepared in iso-octane. Calibration curves were fitted by a second-order polynomial, which was forced through zero. For each sample the concentrations of all individual congeners, CBs 28, 52, 101, 118, 138, 153 and 180 (total concentration denoted as Σ7CB), and CBs 31, 105 and 156 were determined.

Quality assurance

Together with each series of sediment samples, a blank sample (empty Soxhlet thimble) and a reference material (RM) (QOR017MS obtained from QUASIMEME, Aberdeen, UK) were analysed. After the measurement of each batch of samples, two control standard solutions containing the various CBs at concentrations of 3.5 and 41.5 ng/ml, respectively, were analysed as unknowns to check the calibration. The latter was accepted if relative deviations of less than 10% from the target values were found. If not, the calibration was carefully checked and, if deemed necessary, the analyses repeated. Throughout the monitoring period, the laboratory also participated in an international intercalibration exercise, the QUASIMEME Proficiency Testing Scheme (PTS), for the analysis of CBs in sediment.

The results of the analyses of the RM were plotted on a control chart based on Z-scores [10,11]. This Z-score is calculated from:

$$Z = \frac{\left(x_i - \bar{x}\right)}{s_b} \qquad (1)$$

where x_i is the value determined for the analyte concentration in the sample, $\bar{x}$ the assigned value and s_b the target standard deviation or total allowable error (TAE). The assigned value is the reference value of the RM for a given determinant. The TAE can either be calculated from the repeated analysis of the reference material [11] or the value can be set according to the needs of the programme [10]. In the present study, we used the TAEs defined by the QUASIMEME PTS, an approach that has been accepted by the OSPAR monitoring programmes for which the data are intended. In this scheme, the Z-scores are also used for the evaluation of the performance of the participating laboratories and $\bar{x}$ is defined as the best estimate of the true concentration, being calculated from the robust mean of the reported results [10]. The TAEs were set at 6.5% for standard solutions and 12.5% for samples. The latter condition allows distinguishing samples with concentrations which differ by 50%, with 95% confidence.

The analytical data of any given batch of samples were accepted as is, if at least 70% of the Z-scores for the CBs in the RM were $<|2|$ and all Z-scores were $<|3|$. With regard to

the PTS, the analytical procedure was considered to be under control if at least 70% of the Z-scores for CBs in sediments in the PTS were <|2|.

Statistical analysis

Today, a variety of tests is available to analyze trends. Since each of these has its own capabilities and underlying assumptions, considerable experience is required to select the appropriate test in each single instance. Testing the various methods was not the scope of this study. We therefore opted to use purpose-built trend-analysis software, the Trend-y-tector (http://www.trendytector.nl). The procedures and protocols, which underlie this programme, were developed by the ICES Working Group on Statistical Aspects of Environmental Monitoring (WGSAEM). The Trend-y-tector is a suite of methods to detect and estimate trends in annual data and is intended to be used by OSPAR working groups in their assessment of monitoring data. For the purpose of this study the two-sided Mann-Kendall was used, with a significance of 5% and a power to detect a trend of 90%. Mann-Kendall is a straightforward and robust method in detecting monotonic (upward or downward) trends, and is largely unaffected by isolated extreme values, and as such recommended by WGSAEM [13].

CB patterns were analysed with linear correlation analysis using the Statistica [12] software.

4.2.3 Results and discussion

Analysis, quality assurance and long-term reproducibility

The GC-ECD method used for the analysis of the sediment samples gave fully satisfactory results. No experimental problems were encountered in the detection and quantification of the individual congeners, which were found to be present in concentrations ranging from 0.1 to 92 ng/g dry weight (see section on CB concentrations below). Typical chromatograms for a marine and an estuarine sample, which represent samples with fairly low and high concentrations, respectively, are given in Figure 4.2.2.

The long-term analytical performance was well within the quality assurance boundaries set at the outset of the study. From the analytical data obtained for the RM, a coefficient of variation (CV) of between 10% (CB 105) and 54% (CB 28) could be calculated. In the first two years, some problems were encountered with CB 28. After these had been

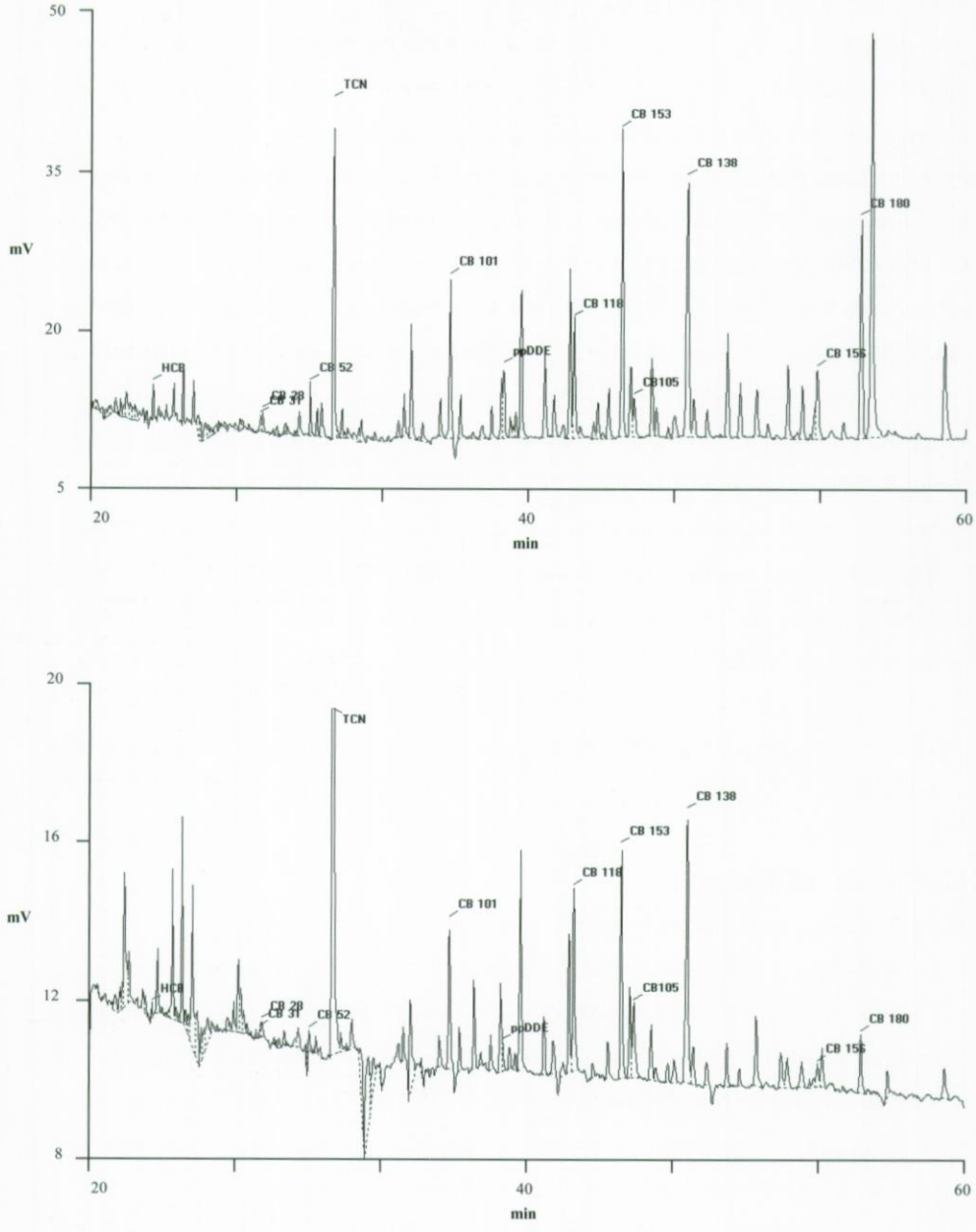

Figure 4.2.2: Typical GC-ECD chromatograms of sediments taken at (A) sampling station S18 in the Scheldt estuary, and (B) station N435 in the North Sea

solved, the results were perfectly satisfactory (CV 18% over 8-year period). Figure 4.2.3 shows Z-scores for the seven marker CBs (28, 52, 101, 118, 138, 153 and 180) in the RM in the period 1993-2002 (*i.e.* after the initial difficulties). As can be read from the graphs, the Z-scores were <|2| in 94% of all cases and never above |3|. In the period during which the samples were analysed (1992 – 2002; analyses were done in the year following the sampling), our laboratory participated in ten QUASIMEME intercomparison exercises for CBs in sediment, which involved the analysis of 19 sediment samples. The Z-scores for the seven marker CBs were <|2| in 77% of all cases. As the allowable error was set at 12.5% by the PTS (*cf.* above), an estimate of the long-term variability could also be calculated from these average Z-scores via Equation 1. This resulted in CVs of between 7% (CB 156) and 35% (CB 31).

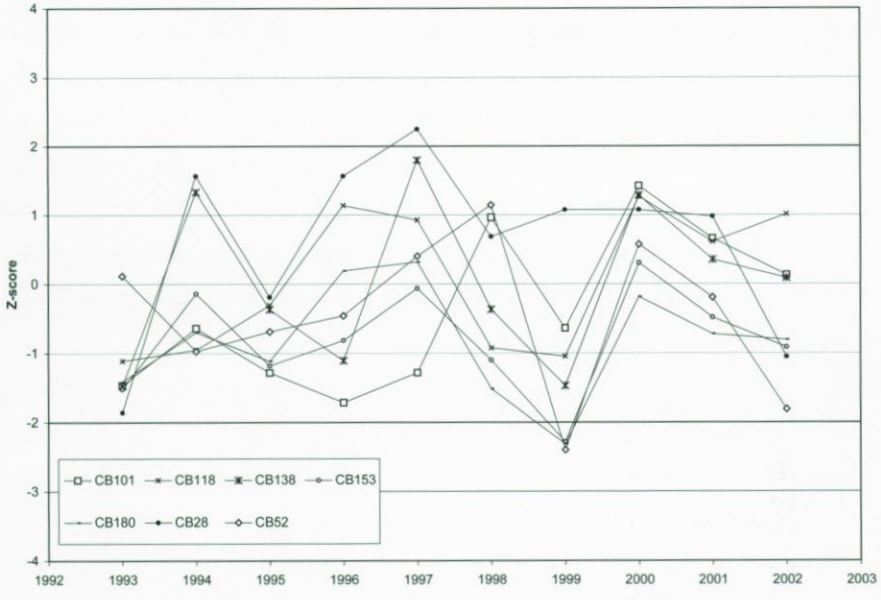

Figure 4.2.3: Z-scores for the seven marker CBs, calculated for the marine sediment QOR017MS used as a reference material, in the period 1993 – 2002.

The CVs obtained in this study, calculated from both the analysis of the RM and participation in a PTS, are at a level that is considered as state-of-the-art for this type of analysis. De Boer and Wells [14] reported CVs between 20 and 33% for CBs in sediments for the first three years of the QUASIMEME interlaboratory study. The general performance did not improve significantly over the next several years. Only a smaller

group of expert labs succeeded in obtaining lower CVs of around 15%. Similarly, during an ICES/IOC/OSPAR intercomparison study held amongst a relatively small group of, mostly, experienced labs, CVs of between 15 and 30% were found [15].

CB concentrations

The influence of the natural variability in sediment composition (grain size, organic matter and mineralogy) has to be taken into account when CB concentrations are compared, *e.g.* to perform a trend analysis or study the spatial distribution. CBs show a much higher affinity to fine particulate matter (containing the bulk of the organic carbon) than to the coarse fraction. The procedure to correct contaminant concentrations for the influence of the natural variability in sediment composition is usually referred to as normalisation [6,16]. For organic contaminants, the organic carbon content (TOC) of the sediment sample is generally used for this purpose. Unfortunately, TOC data were not available for the entire dataset. However, a distinct relation between the organic carbon content and the FS fraction of the sediment in the study area has been demonstrated [17,18]. Alternatively, isolation of the fine fraction by sieving (*e.g.* <20 μm or <63 μm) can be regarded as a physical normalisation to reduce the differences in sediment granulometric composition and can be used for both metals and organic contaminants [19,20]. Sieving removes the coarse particles, which usually do not bind anthropogenic contaminants and dilute their concentration.

Normalising is extremely important for the present dynamic study area. The dynamics of this region are such that the CVs of the fine (<63 μm) fraction range from 20 to 140% (n=17), depending on the station selected. Furthermore, the grain-size distribution of the sediments differs significantly between the different sampling stations and ranges from 1 to 55% fines in the total sediment. The effect is clearly illustrated in Figure 4.2.4 where the data collected in 2001 have been taken as an example. Here, the Σ7CB concentrations (μg/kg dw), expressed for the total sediment (TS) samples are compared with those for the <63 μm fraction (FS). It is evident that differences in grain-size composition of the sediments yield quite different profiles for TS as compared to FS. One illustrative example is provided by the S18 and S22 stations close to Antwerp: while the TS-based data suggest a dramatically different level of contamination (26 *vs* 2.6 μg/kg dw), the FS-based results – with their closely similar 56 and 51 μg/kg dw – show this impression to be incorrect. Another example is the huge difference between the low TS-based

concentrations (0.3–2.6 µg/kg dw) in the estuary and out in a NW direction into the North Sea and the significantly higher values of, frequently, 20–25 µg/kg dw for the fine fraction. In other words, sieving is a powerful step towards normalisation.

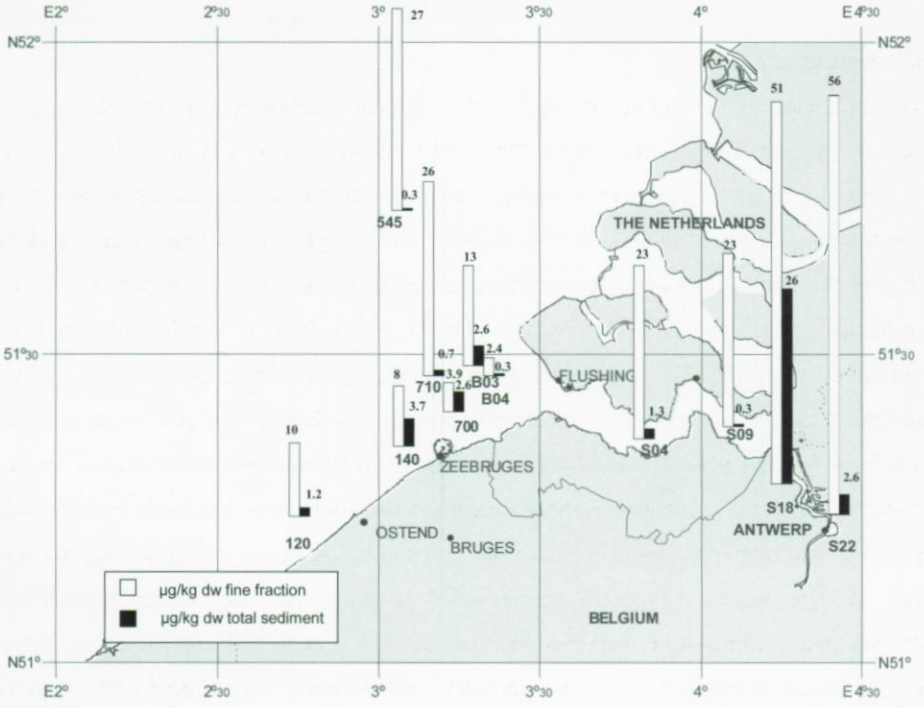

Figure 4.2.4: Σ7CB (µg/kg dw) pattern for sediment samples collected at selected sampling stations in 2001, for (open rectangles) the fine fraction, and (black rectangles) the total sediment.

For the above reasons, analyses were always performed on the <63 µm fraction, and CB concentrations are presented for this fraction unless otherwise specified. As an illustration of the data that were obtained in the ten-year campaign - and which are available as concentrations of the individual CBs for each station and in each year at www.mumm.ac.be - a representative set is shown in Table 4.2.1. For all ten test analytes and Σ7CB, the minimum and maximum concentrations as well as the median value are presented. Related information on %FS is also included. If, for any reason, CB concentrations in the total sediment have to be calculated, one should multiply the recorded values by the%FS/100. The CB concentrations vary from 0.01 µg/kg dw for several CBs (notably CBs 105 and 156) at stations such as N435 and N330, to values of

Table 1: Minimum (Min), maximum (Max) and median (bold) concentration of CBs in the period 1991-2001 in the<63 µm fraction at all sampling stations*.

Station	%<63µm	CB101	CB105	CB118	CB138	CB153	CB156	CB180	CB28	CB31	CB52	SUM7CB
S22	4.7	9.2 - 18	1.9 - 4.7	7.1 - 12	10 - 38	12 - 34	1.0 - 3.7	6.4 - 29	0.7 - 4.4	1.8 - 6.6	4.9 - 8.8	54 - 143
		12	**3.1**	**9.1**	**15**	**17**	**1.5**	**10**	**3.3**	**3.5**	**7.3**	**74**
S18	51	6.6 - 65	0.01 - 18	5.0 - 43	6.5 - 61	7.6 - 92	0.5 - 7.3	2.0 - 49	1.2 - 31	1.2 - 23	2.7 - 42	33 - 382
		9.6	**2.4**	**7.0**	**12**	**13**	**1.6**	**8.7**	**2.6**	**2.0**	**4.6**	**57**
S15	14	6.7 - 20	1.7 - 5.5	5.3 - 12	7.3 - 31	9.3 - 30	0.9 - 3.2	5.6 - 25	1.8 - 7.3	1.4 - 4.3	3.9 - 11	41 - 136
		9.0	**2.3**	**5.8**	**13**	**13**	**1.2**	**6.8**	**2.6**	**2.0**	**5.1**	**59**
S12	7.1	6.5 - 11	0.01 - 3.4	4.9 - 7.9	6.9 - 15	7.8 - 15	0.5 - 1.3	2.1 - 9.3	0.9 - 3.4	1.1 - 2.3	0.6 - 5.5	33 - 66
		8.1	**1.8**	**5.8**	**9.0**	**11**	**1.0**	**6.2**	**2.4**	**1.5**	**3.8**	**48**
S09	1.2	1.4 - 20	0.5 - 6.4	1.0 - 15	1.5 - 34	1.9 - 32	0.01 - 3.0	0.8 - 24	0.08 - 8.1	0.2 - 4.3	0.5 - 9.9	7.2 - 143
		4.2	**1.0**	**3.0**	**5.8**	**5.2**	**0.5**	**2.3**	**0.9**	**0.7**	**1.4**	**24**
S07	3.4	2.5 - 8.0	0.9 - 3.6	2.4 - 7.7	4.2 - 12	3.8 - 11	0.3 - 1.1	2.8 - 5.4	0.9 - 2.7	0.6 - 2.2	1.2 - 4.3	18 - 50
		6.5	**1.2**	**3.9**	**6.1**	**8.1**	**0.7**	**4.4**	**2.3**	**1.7**	**3.8**	**36**
S04	5.6	0.9 - 8.8	0.4 - 4.4	0.8 - 8.9	1.0 - 13	1.1 - 13	0.2 - 1.8	0.4 - 6.3	0.10 - 5.0	0.2 - 3.1	0.3 - 4.6	4.7 - 59
		2.9	**0.8**	**2.3**	**3.4**	**3.5**	**0.4**	**1.8**	**0.8**	**0.7**	**1.3**	**16**
S01	10	0.6 - 3.5	0.3 - 1.3	0.8 - 3.5	1.4 - 6.6	1.1 - 5.0	0.1 - 1.6	0.9 - 3.4	0.7 - 3.0	0.5 - 2.5	0.10 - 2.3	5.9 - 25
		1.3	**0.7**	**1.5**	**1.8**	**2.0**	**0.6**	**1.2**	**0.7**	**0.7**	**0.8**	**8.9**
B10	45	0.9 - 1.7	0.3 - 0.7	0.6 - 1.5	0.8 - 2.2	0.7 - 2.3	0.2 - 0.2	0.2 - 0.8	0.2 - 0.4	0.2 - 0.6	0.4 - 6.0	3.8 - 15
		1.4	**0.6**	**1.4**	**1.8**	**1.9**	**0.2**	**0.6**	**0.3**	**0.3**	**0.7**	**8.3**
B08	26	0.9 - 4.8	0.01 - 1.9	1.0 - 4.6	0.03 - 6.9	1.5 - 6.4	0.01 - 3.1	0.3 - 5.2	0.5 - 4.1	0.3 - 2.9	0.4 - 3.9	6.4 - 30
		3.1	**0.7**	**2.6**	**3.3**	**4.2**	**0.4**	**2.2**	**1.0**	**1.2**	**2.3**	**20**
B07	25	1.7 - 1.9	0.4 - 0.9	1.3 - 1.9	1.7 - 2.6	1.8 - 2.5	0.1 - 0.3	0.6 - 1.0	0.5 - 0.6	0.4 - 0.5	0.7 - 2.0	8.6 - 11
		1.7	**0.5**	**1.5**	**2.1**	**2.4**	**0.2**	**0.9**	**0.5**	**0.4**	**0.9**	**10**
B04	11	0.6 - 2.7	0.01 - 2.1	0.4 - 2.3	0.4 - 3.0	0.4 - 3.0	0.01 - 3.1	0.1 - 3.6	0.1 - 1.2	0.1 - 1.0	0.2 - 3.2	2.3 - 18
		1.5	**0.4**	**1.2**	**1.4**	**1.5**	**0.1**	**0.4**	**0.4**	**0.5**	**1.1**	**8.2**
B03	20	0.3 - 4.2	0.01 - 2.4	0.3 - 4.2	0.2 - 6.3	0.2 - 5.0	0.01 - 3.00	0.01 - 4.3	0.1 - 0.8	0.1 - 0.9	0.3 - 1.7	1.2 - 24
		0.7	**0.2**	**0.4**	**0.5**	**0.6**	**0.5**	**1.0**	**0.4**	**0.3**	**0.5**	**4.6**
N800	2.6	1.5 - 3.1	0.8 - 0.8	1.4 - 2.8	1.9 - 2.4	1.6 - 3.1	0.2 - 0.2	0.5 - 1.2	0.5 - 4.8	0.4 - 7.9	0.7 - 4.2	8.1 - 21
		2.3	**0.8**	**2.8**	**2.4**	**3.1**	**0.2**	**1.2**	**4.0**	**5.0**	**4.2**	**8.1**
N780	35	1.3 - 3.2	0.01 - 1.5	1.2 - 3.3	1.2 - 5.7	1.8 - 5.0	0.01 - 2.1	0.3 - 3.5	0.5 - 4.2	0.4 - 6.6	0.7 - 3.7	7.1 - 23
		2.3	**0.8**	**2.2**	**2.7**	**2.7**	**0.4**	**0.9**	**0.6**	**0.9**	**0.9**	**12**
N710	2.6	1.5 - 8.1	0.01 - 2.5	1.4 - 7.4	1.8 - 7.9	2.0 - 9.1	0.01 - 1.2	0.4 - 4.8	0.4 - 3.9	0.1 - 6.5	0.5 - 3.9	8.7 - 42
		2.2	**0.6**	**2.0**	**2.3**	**2.3**	**0.2**	**0.8**	**0.7**	**0.6**	**0.9**	**13**
N700	66	0.3 - 3.3	0.01 - 1.2	0.4 - 2.7	0.5 - 4.4	0.5 - 4.4	0.06 - 0.5	0.1 - 2.7	0.1 - 4.7	0.2 - 8.6	0.2 - 3.9	2.5 - 20
		1.1	**0.4**	**1.0**	**1.2**	**1.1**	**0.1**	**0.4**	**0.3**	**0.4**	**0.4**	**5.2**
N545	1.0	1.9 - 7.3	1.0 - 5.6	1.8 - 10	1.7 - 12	1.7 - 10.0	0.2 - 1.5	0.4 - 5.0	0.4 - 3.8	0.08 - 6.4	0.7 - 8.7	8.5 - 45
		3.8	**1.3**	**3.6**	**4.0**	**3.5**	**0.3**	**1.0**	**0.7**	**0.6**	**1.4**	**23**
N435	0.9	1.6 - 3.6	0.01 - 2.2	0.01 - 4.4	1.9 - 5.2	1.7 - 4.0	0.01 - 0.5	1.0 - 1.3	0.01 - 0.9	0.01 - 0.7	0.01 - 0.9	6.4 - 19
		2.6	**1.4**	**2.9**	**3.4**	**3.0**	**0.2**	**1.0**	**0.5**	**0.4**	**0.8**	**15**
N421	0.9	1.8 - 4.4	0.5 - 2.2	1.6 - 5.5	2.1 - 5.0	2.3 - 4.2	0.2 - 0.6	0.8 - 2.1	0.4 - 5.4	0.7 - 8.9	1.1 - 4.5	11 - 23
		3.7	**1.1**	**3.9**	**4.3**	**3.7**	**0.3**	**1.5**	**1.3**	**1.4**	**1.9**	**22**
N330	1.3	2.0 - 4.9	0.01 - 6.4	1.7 - 8.4	0.9 - 13	1.6 - 8.5	0.01 - 0.90	0.01 - 3.0	0.5 - 5.1	0.4 - 8.7	0.01 - 4.4	6.9 - 39
		3.1	**0.7**	**2.5**	**2.6**	**2.9**	**0.2**	**1.3**	**0.8**	**0.4**	**0.9**	**22**
N315	1.3	1.3 - 4.9	0.7 - 2.3	1.5 - 4.4	2.3 - 7.0	2.3 - 6.2	0.2 - 0.8	1.0 - 3.9	0.6 - 2.1	0.5 - 2.6	0.6 - 2.2	10 - 28
		3.2	**0.9**	**2.8**	**3.8**	**3.7**	**0.3**	**1.9**	**1.5**	**1.4**	**2.0**	**20**
N250	2.3	1.2 - 4.2	0.5 - 3.5	1.5 - 5.9	2.2 - 12	2.3 - 8.2	0.08 - 0.8	1.0 - 3.3	0.9 - 2.9	0.7 - 2.5	0.4 - 2.7	9.6 - 39
		2.3	**1.1**	**2.3**	**2.8**	**2.8**	**0.3**	**1.3**	**1.4**	**1.6**	**1.4**	**14**
N230	6.2	1.1 - 3.0	0.01 - 0.9	1.5 - 3.0	2.0 - 4.2	2.1 - 4.6	0.01 - 0.5	0.5 - 2.8	0.5 - 2.1	0.6 - 2.9	0.5 - 2.0	9.5 - 20
		2.0	**0.5**	**1.9**	**2.3**	**2.7**	**0.2**	**1.2**	**0.9**	**0.8**	**1.0**	**12**
N150	9.5	0.8 - 3.8	0.01 - 1.0	0.9 - 2.7	0.8 - 3.3	1.2 - 3.8	0.01 - 0.9	0.2 - 2.4	0.5 - 1.9	0.4 - 2.8	0.6 - 14	4.9 - 25
		2.0	**0.6**	**2.0**	**2.2**	**2.3**	**0.3**	**0.8**	**0.6**	**0.7**	**0.8**	**10**
N140	46	0.6 - 3.2	0.01 - 1.9	0.9 - 4.4	0.7 - 6.5	1.2 - 6.5	0.01 - 0.6	0.2 - 3.2	0.2 - 2.8	0.2 - 2.1	0.5 - 2.6	5.5 - 29
		1.6	**0.6**	**1.6**	**1.8**	**1.9**	**0.2**	**0.8**	**0.5**	**0.4**	**0.7**	**8.2**
N120	13	1.1 - 4.9	0.2 - 2.0	1.3 - 4.8	0.7 - 6.5	1.9 - 7.3	0.2 - 1.8	0.6 - 5.2	0.4 - 1.5	0.4 - 2.0	0.3 - 5.5	7.6 - 30
		2.1	**1.0**	**1.9**	**2.8**	**2.6**	**0.3**	**1.2**	**0.8**	**0.6**	**0.8**	**12**
N115	11	1.7 - 2.5	0.8 - 1.7	1.8 - 3.6	2.3 - 6.7	2.3 - 4.8	0.08 - 0.3	1.2 - 3.0	0.8 - 2.5	0.5 - 2.0	0.8 - 1.5	11 - 25
		2.1	**1.2**	**2.7**	**4.5**	**3.6**	**0.2**	**2.1**	**1.7**	**1.3**	**1.2**	**18**

* The number of samples analysed at each station typically was between 2 and 12.

24–92 µg/kg dw for, specifically, CBs 138, 153 and 180 at stations such as S18 and S22. Median values vary from, frequently, 0.1–0.3 µg/kg for CB 156 to occasional highs of 12–17 µg/kg dw for CBs 138 and 153. There is a clear gradient for essentially all the congeners from the city of Antwerp to Flushing (stations S22–S01), as can be read most easily from the median Σ7CB concentrations which decrease from (60 ± 10) µg/kg dw in the vicinity of the former city to values of 10 µg/kg dw or somewhat less, close to the North Sea. It is good to add that, geographically, the mouth of the estuary is situated at Flushing but, geomorphologically, it extends from Zeebruges to the Island of Walcheren [21]. It is precisely in this area that mud deposits are formed, which may explain the observed lower levels. This aspect will be discussed in more detail below.

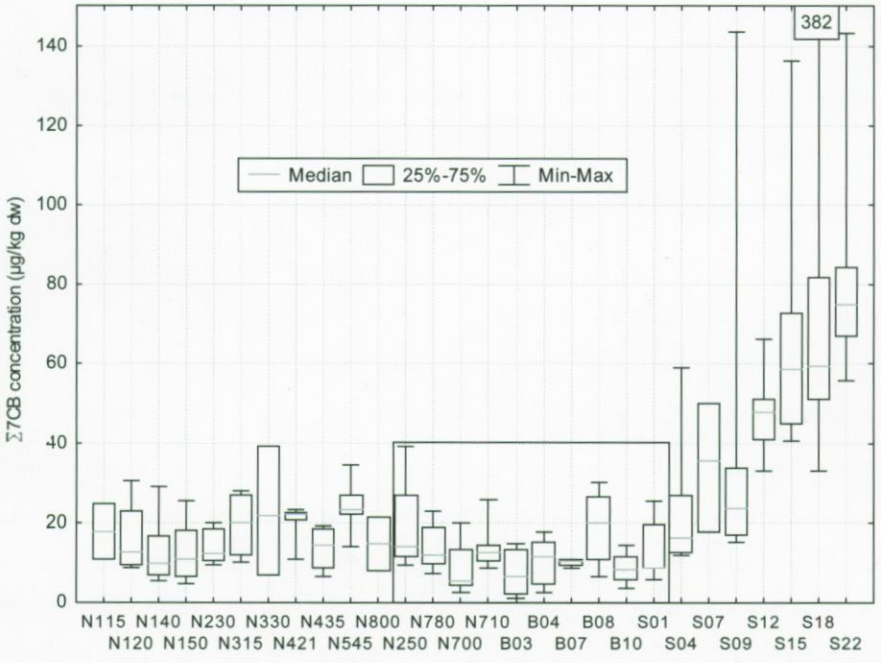

Figure 4.2.5: Concentrations of Σ7CB (µg/kg dw) in fine sediment (< 63 µm) for the period 1991-2001 (n=10-100). The rectangle comprises the stations in the mouth of the Scheldt estuary.

In Figure 4.2.5 the Σ7CB concentrations for the entire dataset are plotted as a box and whisker plot. The plot illustrates the gradient and also gives a good impression of the year-to-year variability of the data by means of the boxes (25–75 percentiles). This variability is much higher than the long-term analytical variability and ranges from 30 to

200%. The Figure also shows that median Σ7CB concentrations in the North Sea are not significantly different from those more inshore, with perhaps two exceptions, the stations N700 near Zeebruges and B03 in the mouth of the estuary. The significantly lower concentrations found at these stations reflect the much more dynamic situation in the estuary. A similar drop in Σ7CB concentrations is also found for the Rhine/Meuse mouth [22] in Dutch coastal waters, where concentrations are about half as large as those in the rest of the Dutch coastal waters. Moreover, the Σ7CB concentrations found in the present study are similar to earlier findings by Delbeke *et al.* [23], Laane *et al.* [24] and recent Dutch data for the same region [22]. Our findings show that the southern North Sea is more polluted than, *e.g.*, the western and central North Sea with their Σ7CB concentrations of less than 2 μg/kg dw [5,22]. Together with the Ems and Elbe estuaries, the Scheldt estuary is among the more polluted areas in the region [5].

Table 4.2.2: Ranges and median concentrations in ng/kg dw of selected CBs for North Sea stations of this study and background concentrations [5].

Compound	This study		Southern Norwegian Sea/Skagerak	Iceland Sea/Norwegian Sea
	Range	Median		
CB 28	0.1 - 1300	150	31	<10
CB 52	0.1 - 1300	220	32	<10
CB 101	15 - 2100	200	62	16
CB 153	8 - 3000	260	90	20
CB 138	16 - 3000	280	116	26
CB 180	0.1 - 1700	100	60	<10

The concentrations found during this study were compared with OSPAR 'background/reference concentrations' (BRC) and 'ecotoxicological assessment criteria' (EAC). These tools were developed to assess the impact of certain micro-contaminants [5] based, in this instance, on TS rather than FS data, and to create target values against which the goals set in the OSPAR strategy for hazardous substances can be evaluated [25]. The objective of the OSPAR Commission with regard to hazardous substances is "to prevent pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances (such as CBs), with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances." For highly persistent and ubiquitous organic pollutants such as CBs, analyte concentrations typical for remote and other selected parts of the OSPAR area are used as BRC. Table 4.2.2 summarises

background concentrations (which, actually, in many cases seem much too precise) reported by OSPAR as low (Icelandic/Norwegian Seas) and distinctly higher (sea off southern Norwegian coast/Skagerak) concentrations, together with ranges and median concentrations of the same CBs found during this study. To this end, the median CB concentrations of Table 4.2.1 were, first, converted into TS concentrations and, next, compared with the BRCs of Table 4.2.2. When the concentrations of all stations were considered, 68% were above the higher and 30% between the higher and the lower BRC. However, the BRCs were defined for the marine environment and, consequently, should not be applied to estuaries. If calculations were limited to the open North Sea, the results, indeed, improved: only 50% of the concentrations were now above the higher, and 46% between the higher and the lower BRC. In other words, 50% of the open-sea data were below the higher threshold level – a result that should eventually be found for all sampling sites. In addition, not only are the BRCs much too precise, they are given for total sediment concentrations and do not consider the sediment composition, specifically the FS content. The experimental results discussed earlier in this paper clearly indicate that comparing sediments (with their, frequently, mutually very different composition) with respect to parameters such as BRCs should be FS- rather than TS-based.

EACs are defined as concentrations below which no harm to the marine environment is expected. OSPAR-derived criteria for the specific contaminants using all available ecotoxicological data that passed predefined quality criteria [5]. The principle of the procedure is the derivation of an extrapolated concentration based on ecotoxicological information. Subsequently, an ecotoxicological assessment criterion is generated by setting an interval around the extrapolated concentration. The extrapolated concentration is calculated by selecting the lowest NOEC or L(E)C50 from the toxicological data available and applying extrapolation factors which depend on the extent of the data set. Specifically for CBs, TEQs were also taken into account. Subsequently, the extrapolated concentration is rounded to the nearest order of magnitude interval to generate the EAC [26]. Ecotoxicological assessment criteria should be used to identify possible areas of concern and indicate which substances could be considered a priority, and should not be used as firm standards or as triggers for remedial action. The provisional EAC for CBs has a lower limit of 1 μg/kg dw and a higher limit of 10 μg/kg dw for Σ7CB. A plot of the median values of Σ7CB found at the different stations in the period 1991-2001 against the EACs is shown in Figure 4.2.6. Sampling station S18 was found to be the only one having

a median concentration that is above the upper EAC level (29 *vs.* 10 µg/kg dw), and harm to living organisms can, therefore, not be excluded. In about half of all the stations, the lower EAC level was exceeded, which makes these stations areas of concern. It is therefore interesting to add that several of these high values were found at stations out in the open North Sea. This is a rather serious result because EAC levels are based on effects. A discussion concerning data normalisation is probably not justified here. Organisms are in contact with the entire sediment and not just the fine fraction. Comparisons on a toxicological basis are therefore best done on the total sediment concentration.

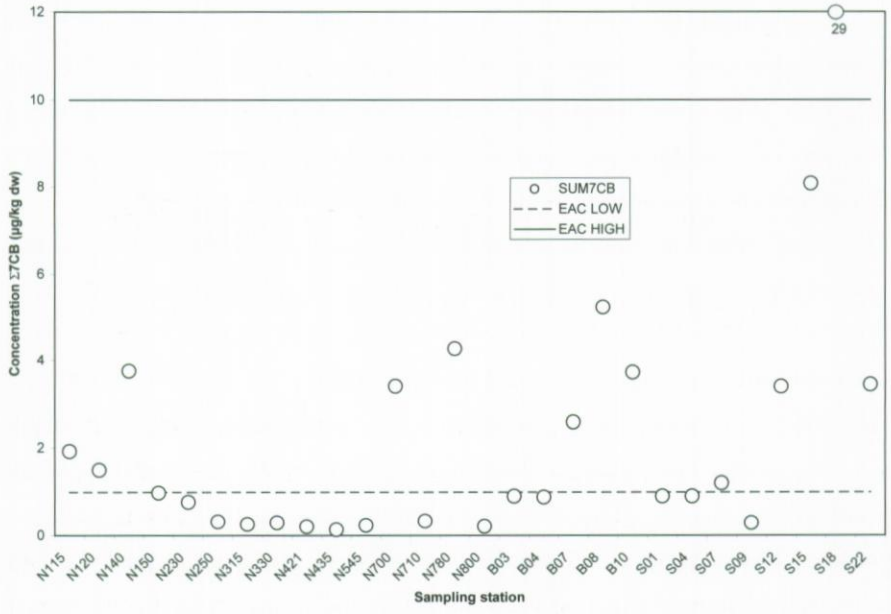

Figure 4.2.6: Median concentrations of Σ7CB (µg/kg dw) in 1991-2001 plotted against the OSPAR EACs [5].

CB patterns

In an earlier paper, Vyncke *et al.* [27] studied the CB patterns in sediments from the Scheldt river and noted that the various CBs significantly correlated with each other when the FS concentration data for a number of years were compared. Earlier, Delbeke *et al.* [23] also found a consistent CB pattern in sediments from the present study area. In this study, this aspect was further investigated not only for the Scheldt, but for all stations.

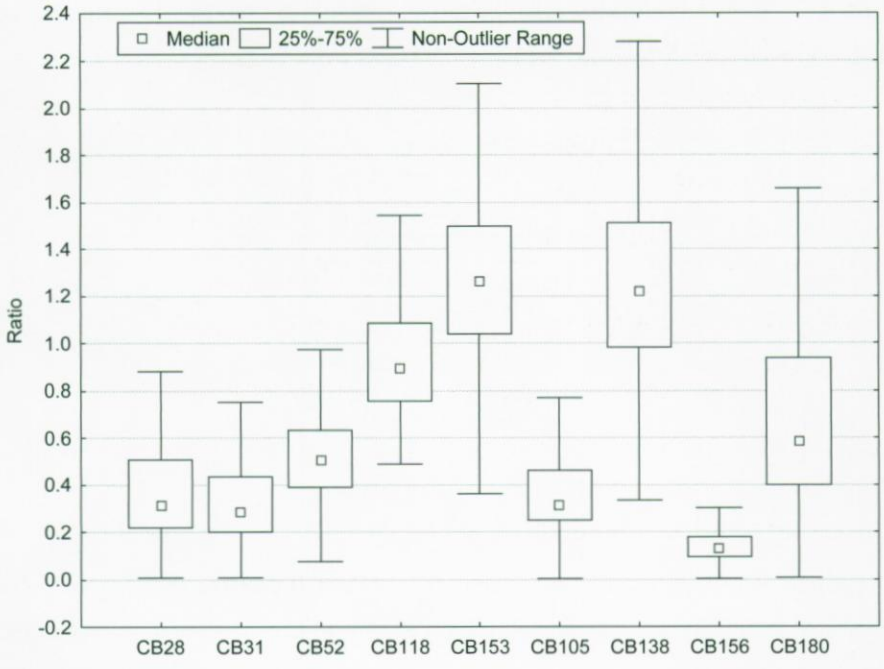

Figure 4.2.7: CBx/CB101 ratios averaged (n=184) over all sampling stations and the entire period (1991-2001). The median value, the 25-75% percentiles and the non-outlier range are indicated.

To this end, the average ratio of each individual CB over CB101 was calculated for all sampling stations and over the entire 10-year study period. The box and whisker plot of Figure 4.2.7 graphically displays the results. The quartiles of the plots vary between 15 and 33% for the various CBx/CB101 ratios, which is of the same order as the long-term analytical variability. The whiskers are roughly similar in length to the long-term analytical error. In other words, the plot certainly suggests that all the stations in the Scheldt estuary and southern North Sea have closely similar CB patterns. This confirms and extends the conclusions quoted above or, at the very least, shows that the pattern variability is less than the long-term analytical variability. As a further demonstration, the logarithms (a log-normal distribution gave the best representation of the population) of the concentrations of the individual CBs were plotted versus the logarithms of the CB101 concentrations for the entire dataset. As Table 4.2.3 shows, significant correlations were found with r^2 values form 0.66 for CB 31 to 0.97 for CB 153. It is interesting to add that such a distinct correlation was also found for another dataset. When we carried out a similar analysis for results published by de Boer *et al.* [28], who studied samples

originating form various marine and estuarine stations in the coastal area of the Netherlands, even slightly higher r^2 values of 0.89-0.98 were found.

Table 4.2.3 : Log-log correlation of various CBs *vs.* CB 101.

CB	r^2
105	0.83
118	0.95
138	0.94
153	0.97
156	0.80
180	0.86
28	0.72
31	0.66
52	0.84

It is tempting to explain the observed similarity in the CB patterns on the basis of a common source. In that case, the Scheldt river is the most likely candidate because of the high concentrations found up-stream and the observed concentration gradient (Figure 4.2.4). However, the similarity in the patterns extends over the entire region under investigation and it remains to be proven that the Scheldt river would influence sampling stations such as N120 and N800 (Figure 4.2.1). Here, one has to consider that the existence of several sources could still result in closely similar patterns, provided that essentially the same mixtures are used, and this is indeed true for CBs. The major constituents of these mixtures have become the marker CBs that are found in all environmental samples [2,32]. Large-scale diffuse contamination of soil and sediments by CBs has been largely attributed to atmospheric transport and deposition [32]. This process is certainly more important for the open seas and exceeds the direct riverine input for the area [5].

It is therefore highly likely that the general use of technical CB mixtures with roughly the same composition is the main cause of the pattern similarity. The complex sediment movements in the North Sea, and transport of suspended matter by major rivers such as the Scheldt and Rhine and in the Channel, in combination with atmospheric transport and deposition, should then mainly be regarded as the mechanisms leading to the widespread distribution of these contaminants. For instance, for the Scheldt there is a significant incursion of marine sediment [29,30], mostly originating from the Channel [21,31], into

the estuary and the residual transport of marine mud into the estuary is in near-equilibrium with the output of fluviatile mud. Only during high flood events, considerable amounts of sediment are transported to the sea, contributing to the net transport [29]. Once at sea, sediments and suspended particulate matter can be redistributed by currents and intensive sediment movements, which occur frequently in that part of the North Sea.

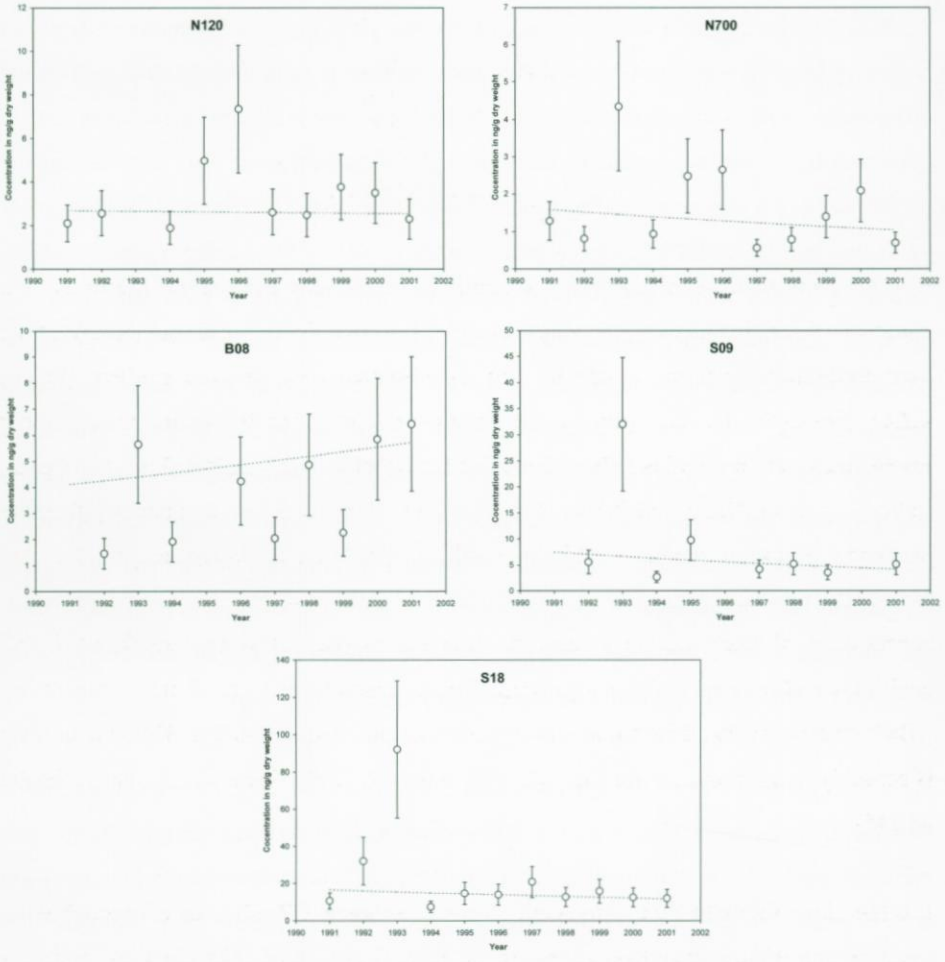

Figure 4.2.8: Trend analysis of CB 153 concentrations for sampling stations N120 (Belgian coast), N700 (deposition area), B08 (mouth of Scheldt estuary), S09 (middle of Scheldt estuary) and S18 (turbidity maximum) in the period 1991-2001. The dashed line is the predicted trend.

Trend analysis

Because of the good correlation of the concentration data for the various marker CBs discussed above, trend analysis was limited to a single - but ubiquitous - congener, CB 153. Five stations were selected for which experimental results were abundantly available, and which gave a good spatial distribution: stations N120 (Belgian coast), N700 (deposition area), B08 (mouth of estuary), S09 (middle of estuary) and S18 (turbidity maximum). The results of the Trend-y-tector analysis are shown in Figure 4.2.8. One main conclusion is that the year-to-year differences are sometimes large, or even very large. It was also observed that, with the marine sampling stations, interannual differences for each station regularly were larger than those between different stations. In other words, the environmental variation is high. Nicholson *et al.* [33] showed that the performance of a temporal trend programme can be measured by the trend (% change per year) that can be detected after ten years with a power of 90% using a test at the 5% significance level with state-of-the-art statistical methods such as were used here. To quote two examples, for a high environmental variability (52%), a trend of 22% can be detected for an analytical variability of 12.5%, and a trend of 24% for an analytical variability of 25%. For a medium variability (26%), a trend of 12 or 15% can be detected for these same analytical variabilities. Furthermore, the statistical method used here is largely unaffected by isolated extreme values. Given our long-term analytical variability (see above) and the expected as well as observed environmental variability, the current programme will not be able to detect changes that are, on average, less than about 20%. Or, in other words, the weak upward (B08) or downward (N700 and S09) trends suggested by the Mann-Kendall analysis certainly are not significant.

With the changes in the CB concentrations being less than 20% during the period of monitoring discussed in this study, one has to conclude that no concentration changes can be indicated[†††]. This is very much in line with the most recent assessment of contaminant data in sediments from the OSPAR area, i.e. the northeast Atlantic [38]. From the 308 time series that were evaluated only fourteen showed a downward trend. Apart from six time series that even showed an upward trend, the vast majority of the time series showed

[†††] It is unlikely that the quoted results is (partially) due to the type of sampling used. Using another type of sampler, e.g. a box corer instead of a grab sampler, will presumably have little effect on the outcome. A box corer is often used in area's with a clear net sedimentation rate, a situation that does not occur here (with possibly the exception of certain zones in the Scheldt estuary; however, there dredging will interfere with the natural processes). That is, it is safe to assume that, in the study area, the upper 10-40 cm do indeed represent the current situation [7b].

that concentrations have not changed in the last 5-10 years. Actually, in recent years few authors have been able to relate decreased inputs of CBs resulting from the reductions in use, discharge and/or emission, to reduced levels in the marine environment. Admittedly, decreasing trends were observed earlier in biota for the southern North Sea [34] and the Baltic [35], and also for sediments in these same areas [24,36]. However, such downward trends were mostly observed in the eighties and early nineties. Today, a rather large number of non-significant trends is reported for the Greater North Sea area [5]. Already in 1988 Tanabe [37] already suggested that, although CB concentrations were decreasing in certain regions, a global decline should not be expected in the next few years, because of continuous inputs into the environment caused by e.g. leakages from landfills and emissions from incinerators. In addition, he even suggested that the quantities of CBs still in use exceed the amount that has been released into the environment. OSPAR states that, for the northeast Atlantic region, the major part of the CBs that are present, is adsorbed to soil, litter and sediment. From these solid matrices they are slowly volatilised to the atmosphere [32]. Such emissions will act as a constant source of supply. Moreover, according to OSPAR, until at least the very end of our study, emissions of CBs from small, uncontrolled applications in the northeast Atlantic were still very important. Clearly, CBs are still being introduced into the marine environment, while at the same time redistribution occurs through various, often complex, processes such as sediment transport. Our results fit into this picture. The net effect is that CB concentrations have remained essentially constant in the past decade – a situation which seems unlikely to change in the near future.

4.2.4 Conclusions

The current analytical methodology for the determination of CBs in sediments by means of GC-ECD gives a long-term variability of 10-30%. This is simply the reality for most laboratories that perform this type of analysis. Given this analytical variability and the expected as well as observed environmental variability, the current programme will not be able to detect changes that are, on average, less than about 20% over a 10-year period. This is the case even though normalising for the fine fraction of the sediments already reduced the overall variability of the data, *i.e.* the variability within a station resulting from different sampling occasions. In this respect, isolation of the fine fraction by sieving (<63 μm) seems an efficient physical normalisation step, if not an indispensable one, for time trend analysis of CBs in sediments. Not taking the grain size into account would

result in differences between the sampling stations purely on the basis of their sediment composition rather that the actual CB levels.

The present results show that the normalised concentration patterns of the seven marker CBs were closely similar in all stations of the study. Although a clear gradient is observed in the estuary leading to the inevitable conclusion that the Scheldt river is the main source, the pattern similarity (especially in the open North Sea) certainly also reflects the well-known fact of the production and widespread use of identical CB mixtures. Common processes in the marine environment such as the complex movements of marine sediment, transport of suspended matter and atmospheric transport quite probably further reduce the source differences.

CB concentrations at about half of all the sampling stations were above the OSPAR background level or BRC. One should add that, although the order of magnitude of the BRCs are correct, the values given are much to precise; moreover they do not consider the sediment composition, specifically the FS content, which severely hampers their use. The lower OSPAR EAC level was also frequently exceeded, again at about half of the stations, and several of the high values were found at stations out in the open North Sea. As EAC levels are based on effects, this is a cause for serious concern.

The CB concentrations were found to have remained essentially constant in the entire study area in the 1991-2001 period *i.e.* changes were less than 20%. The ten years of monitoring represent a considerable investment in time and money, and a brief critical assessment of the merits of the programme is therefore justified. In all probability, being able to detect a, hopefully downward, trend of about 3% per year will be considered satisfactory for many purposes. This implies that a monitoring programme of the present size should be continued. It is good to add that the perceived absence of a distinct trend should not lead the authorities to replace annual monitoring by e.g., monitoring every two or three years. Bignert *et al.* [35] have already demonstrated that reduced data sets easily leads to erroneous results *i.e.* non-justified upward or downward trends. Actually, performing such an exercise on our present dataset, led to the same conclusions.

Finally, should there be a need to improve the performance of the programme without significantly increasing costs, then one option would be to reduce the number of stations

and proportionally increase the sampling frequency at the remaining stations. After all, the present study convincingly demonstrates the close similarity of the CB patterns in the entire study area, and also the not too widely divergent concentrations in the sediments from the open sea. An alternative would be to use SPM for the measurements

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Samenvatting

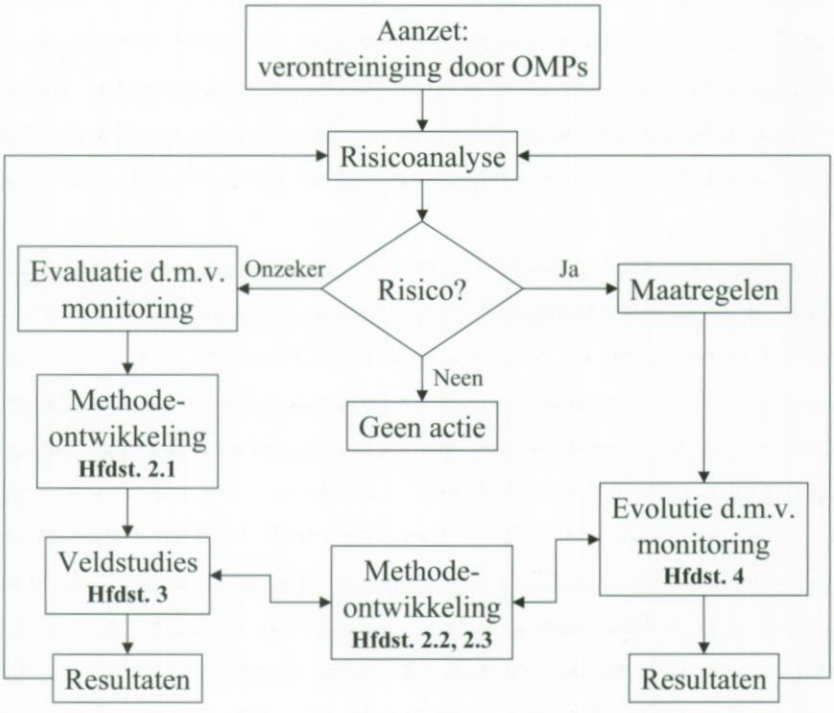
Bij de evaluatie van de effecten van menselijke activiteiten op het mariene milieu speelt monitoring een belangrijke rol. Dit is speciaal zo wanneer de gevaren van zogenaamde toxische stoffen in beschouwing genomen worden. Onder monitoring wordt hierbij verstaan het geregeld meten van de concentraties van die stoffen – in deze studie vluchtige organische verbindingen (VOCs) en polygechloreerde bifenylen (PCBs) – in de verschillende compartimenten van dit milieu. Dit maakt het mogelijk om enerzijds de bestaande situaties in kaart te brengen en anderzijds veranderingen in die situatie vast te stellen. Het laatste is ondermeer van belang om de efficiëntie van genomen maatregelen te toetsen (bijv. het verbod om bepaalde bestrijdingsmiddelen te gebruiken in de landbouw) en/of aan te passen. De activiteiten van een laboratorium voor mariene chemie bevinden zich precies in dit gebied en drie voorname aspecten van dit werk worden in deze studie toegelicht:

- de ontwikkeling en verbetering van analytische procedures die in staat moeten zijn om sporen van organische micro-contaminanten in het mariene milieu te bepalen
- het uitvoeren van baseline studies om de potentiële impact van "nieuwe" contaminanten te evalueren
- het observeren en evalueren van de aanwezigheid van een doelgroep van contaminanten in het mariene milieu in ruimte en tijd.

De algemene aanpak van de riscoanalyse van schadelijke stoffen is grafisch voorgesteld in Figuur 1. De bijdragen hieraan geleverd door de auteur van de hudige studie zijn eveneens aangegeven.

Hoofdstuk 1 schetst de aanzet voor en het huidige kader van de monitoring van het mariene milieu. De aanzet voor zowel nationale als internationale monitoring programma's is een gevolg van de erkenning van de verontreiniging door organische micropolluenten (OMPs) als een mondiaal probleem. Het hoofdstuk beschrijft kort een aantal belangrijke regionale programma's zoals HELCOM (Oostzee), AMAP (Noordpoolgebied) en mondiale programma's zoals UNEP-POP (de Stockholm Conventie), RSP (Regional Seas Programme) en GIWA (Global International Waters Assessment), en gaat dieper in op programma's die van belang zijn voor de Noordzee,

met name: het '*Joint Monitoring and Assessment Programme*' van OSPAR (OSPAR JAMP) en de kaderrichtlijn water (KRW) van de EU. Hoewel hij pas recent werd goedgekeurd, kan verwacht worden dat deze richtlijn in de komende jaren een belangrijke rol zal spelen. Het hoofdstuk gaat ook dieper in op de strategie gebruikt voor het prioritiseren van gevaarlijke stoffen en besteedt aandact aan stoffen en stofklassen die belangrijk zijn, of worden, voor de Noordzee en haar directe omgeving. Verder worden de voornaamste tekortkomingen van de monitoringsprogramma's benoemd en worden er voorstellen tot verbetering geformuleerd. Tot slot gaat de tekst kort in op de meest recente methodologie inzake monsteropwerking en instrumentele analyse (in hoofdzaak gas- en vloeistofchromatografie), waarbij zowel de state-of-the-art als veelbelovende nieuwe initiatieven worden toegelicht.



Figuur 1: Algemene aanpak en activiteiten in monitoring studies gericht op de risicoanalyse van OMPs, inclusief de bijdragen die hiervoor geleverd werden door de auteur van dit proefschrift.

Analyse

Hoewel de laatste jaren veel aandacht is besteed aan het prioritiseren van OMPs – wat resulteerde in de publicatie van een aanzienlijk aantal lijsten – blijkt vaak dat informatie inzake de aanwezigheid en concentraties in het mariene milieu voor veel OMPs voorkomend op deze lijsten, schaars is. De VOCs zijn in dit opzicht een goed voorbeeld. De noodzaak om zowel de aanwezigheid als de concentraties van deze verbindingen te bepalen, leidde tot de ontwikkeling van een analytische GC–MS methode die de gelijktijdige bepaling van chloroform, tetrachloromethaan, 1,1-dichloroethaan, 1,2-dichloroethaan, 1,1,1-trichloroethaan, trichloroetheen, tetrachloroetheen en de BTEX verbindingen in mariene organismen mogelijk maakt. **Hoofdstuk 2.1** laat zien hoe een commercieel '*purge-and-trap*' apparaat hiertoe werd aangepast en gaat dieper in op de problematiek van dit soort analyse. Bijzondere aandacht wordt besteed aan verontreininging door omgevingscondities en de robuustheid van de methode in het algemeen. Bovendien wordt beschreven hoe de methode met succes werd gebruikt voor het bepalen van VOCs in twee soorten Noordzeevis. Bijkomende technische verbeteringen en het gebruik van een meer geschikte GC kolom hebben er uiteindelijk toe geleid dat de methode gebruikt kan worden voor ongeveer zestig VOCs (**Hoofdstuk 2.2**).

Het tempo waarin nieuwe analytische instrumenten op de markt komen, is in grote mate bepalend voor de snelheid waarmee de analytische chemie evolueert. Een laboratorium voor mariene chemie is dan ook genoodzaakt om deze evolutie van dichtbij te volgen en, indien gewenst, nieuwe analysemethoden te implementeren. Een goed voorbeeld hiervan wordt beschreven in **Hoofdstuk 2.3**. Het behandelt de evaluatie van een nieuw tafelmodel '*high-resolution time-of-flight mass spectrometer*' (TOF MS) voor de bepaling van een aantal belangrijke OMPs. Het voornaamste voordeel van dit instrument is zijn hoge massaresolutie van ca. 0,002 Da (10 ppm); dat verhoogt de detecteerbaarheid van verbindingen zoals polaire pesticiden, polyaromatische koolwaterstoffen en PCBs in aanzienlijke mate. De detectiegrenzen die nu bereikt kunnen worden, zijn excellent, d.w.z. ca. 1-4 pg geïnjecteerde massa. Vooral voor de analyse van complexe monsters biedt het instrument belangrijke voordelen vergeleken met klassieke lage-resolutie MS-systemen, omdat de kans op vals-positieve resultaten aanzienlijk wordt verkleind.

Baseline monitoring

Het zich bewust worden van (het voorkomen) en het mogelijke gevaar van bepaalde verbindingen is dikwijls de voornaamste aanzet voor de ontwikkeling van nieuwe analytische methoden. Als die eenmaal operationeel zijn, kunnen ze gebruikt worden voor een eerste '*baseline*' studie van de concentraties in het milieu. Dit maakt het de autoriteiten mogelijk een eerste inschatting te maken van de omvang van het probleem. De initiële resultaten voor VOCs beschreven in Hoofdstuk 2.2, laten concentratiesniveaus in beide vissoorten zien die tot 100-maal hoger waren dan in het omliggende water. Bovendien waren de hoogste concentraties ongeveer tienmaal hoger dan voor CB 153, de CB die normaliter met de hoogste concentratie aangetroffen wordt. Het directe gevolg was het initiëren van een meer uitgebreid vierjarig onderzoek in de zuidelijke Noordzee: dit wordt besproken in **Hoofdstuk 3.1**. Tijdens opeenvolgende campagnes werden twee soorten vis en vier soorten invertebraten bemonsterd. De eerdere resultaten werden bevestigd: VOCs konden worden aangetoond in alle soorten en de concentraties waren van dezelfde orde van grootte als in het eerdere onderzoek. Verder bleken de concentraties van de gechloreerde VOCs, met uitzondering van chloroform, in het algemeen lager te zijn dan die van de BTEX verbindingen. Bovendien konden de verspreidingspatronen en concentraties van BTEX in verband gebracht worden met hun voornaamste bron, de verbranding van fossiele brandstoffen. Met gebruikmaken van relevante veiligheidscriteria kon worden vastgesteld dat de concentraties in de onderzochte diersoorten geen acuut gevaar opleveren voor de organismen. Ook ziet het er naar uit dat consumptie van deze organismen geen gevaar oplevert voor de mens. Verder onderzoek is evenwel nodig om te bepalen wat de gevolgen zijn van een langdurige blootstelling aan lage concentraties. Dat sediment niet als een bron van VOCs beschouwd kan worden, komt duidelijk naar voren in **Hoofdstuk 3.2**. De concentraties in organismen konden niet gerelateerd worden aan de extreem lage concentraties in het sediment. Dit suggereert dat organismen VOCs voornamelijk via de waterkolom opnemen. Opvallend waren tijdens dit onderzoek ook de, soms verontrustend, hoge VOC concentraties van sommige micro-polluenten (tot 900 pg/g versgewicht) in de buurt van de haven van Antwerpen.

Het bovenstaande laat niet alleen zien dat VOCs aangetoond kunnen worden in mariene organismen, maar ook dat zulke organismen geschikt kunnen zijn om de verontreiniging van het mariene milieu in kaart te brengen. De hoge concentraties gevonden in aal in de

buurt van Antwerpen (Hoofdstuk 3.2) roepen onmiddellijk vragen op over de situatie in het zoetwatermilieu. In **Hoofdsuk 3.3** wordt daarvan een verkennende studie beschreven over de aanwezigheid van VOCs in een aantal zoetwatermilieus in Vlaanderen en wordt het gebruik van aal als biomonitor geëvalueerd. De concentraties in aal blijken representatief te zijn voor hun 'milieu' en de VOC patronen en concentraties gevonden in vissen afkomstig van dezelfde locatie blijken vergelijkbaar te zijn. Bovendien blijken de concentraties in de waterkolom en de palingen modelmatig met elkaar overeen te komen. Ook in dti geval konden de experimenteel bepaalde concentraties en patronen van BTEX in verband gebracht worden met de verbranding van fossiele brandstoffen.

Compliance monitoring

Wanneer we de studie van het mariene milieu in zijn totaliteit beschouwen, dan behoort het werk beschreven in de voorgaande hoofdstukken tot het zogenaamde verkennend onderzoek –methodeontwikkeling, de eerste reeksen metingen en de eerste resultaten. Aansluitend hierop is het interessant om in het laatste deel van dit proefschrift dieper in te gaan op de resultaten van lange-termijn monitoring. In **Hoofdstuk 4.1** wordt de evolutie van de concentraties van CBs in vier indicatorsoorten, kabeljauw, bot, mossel en garnaal, in een periode van 10 jaar (1983–1993) besproken. De studie gaat ook in op de relatie tussen die concentraties en een aantal biologische parameters. Zo wordt aangetoond dat het nodig is om te normaliseren op vetgehalte om tot een verantwoorde vergelijking van CB gehalten in verschillende organismen binnen een soort en/of tussen verschillende weefsels van één organisme te komen. Het onderzoek laat ook zien dat de concentraties van CBs in het Belgische gedeelte van de Noordzee in de periode van onderzoek 40–70 % gedaald zijn. In **Hoofdstuk 4.2** wordt een vergelijkbare studie beschreven over de CB gehalten van sedimenten uit hetzelfde gebied, maar nu voor de periode 1991–2001. De studie gaat ook in op de evaluatie van de lange-termijn kwaliteit van de analytisch-chemische bepalingen en toont aan dat de CB patronen in de fijne fractie van het sediment (<63 µm) vergelijkbaar zijn voor het gehele onderzochte gebied. Het isoleren van die fractie door middel van zeven kan beschouwd worden als een fysische normalisatie van het sediment: verschillen in concentratie als gevolg van variaties in de granulometrische samenstelling van het sediment worden grotendeels geëlimineerd. Daardoor wordt een beter inzicht in de verspreiding en de patronen van de CBs verkregen en kan een betere trendanalyse worden uitgevoerd. Het is enigzins opmerkelijk dat, waar de concentraties van CBs in organismen in Hoofdstuk 4.1 een duidelijke afname vertoonden, er hier, voor

het sediment en voor een meer recente periode, geen significante dalende trend is vast te stellen. Doordat het om verschillende perioden en verschillende matrices gaat, dient men voorzichtig te zijn met het trekken van conclusies. Desalniettemin is het interessant om te vermelden dat ook ander recent onderzoek van biota en sediment constateert dat er geen duidelijke concentratiedalingen zijn.

List of publications

This thesis is based on the following papers which have been published or submitted as regular contributions to scientific journals:

P. Roose and U. A. Th. Brinkman, Determination of volatile organic compounds in marine biota. *J. Chromatogr. A*, 799 (1998) 233-248. (**Chapter 2.1**)

P. Roose, K. Cooreman and W. Vyncke, PCB's in cod (*Gadus morhua*), flounder (*Platichtys flesus*), blue mussel (*Mytilus edulis*) and brown shrimp (*Crangon crangon*) from the Belgian Continental Shelf: relation to biological parameters and trend analysis. *Chemosphere*, 37 (1996) 2199-2210. (**Chapter 4.1**)

P. Roose and U. A. Th. Brinkman, Improved determination of VOCs in marine biota using on-line purge and trap-gas chromatography-mass spectrometry. *The Analyst*, 123 (1998) 2167-2173. (**Chapter 2.2**)

P. Roose and U. A. Th. Brinkman, Volatile organic compounds in various marine organisms from the southern North Sea. *Mar. Poll. Bull.*, 35 (2001) 1478-1488. (**Chapter 3.1**)

P. Roose, J. Dewulf, U. A. Th. Brinkman, and H. Van Langenhove, Measurement of volatile organic compounds in sediments of the Scheldt estuary and the southern North Sea. *Water Res.*, 35 (2001) 1478-1488. (**Chapter 3.2**)

J. Dallüge, P. Roose and U. A. Th. Brinkman, Evaluation of a high-resolution time-of-flight mass spectrometer for the gas chromatographic determination of selected environmental contaminants. *J. Chromatogr. A*, 970 (2002) 213-223. (**Chapter 2.3**)

P. Roose, G. Van Thuyne, C. Belpaire, M. Raemaekers and U. A. Th. Brinkman, Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium). *J. Environ. Monit.*, 5 (2003) 876-884. (**Chapter 3.3**)

P. Roose, M. Raemaekers, K. Cooreman and U. A. Th. Brinkman, Polychlorinated biphenyls in marine sediments from the southern North Sea and Scheldt estuary: a ten-year study of concentrations, patterns and trends. Submitted to *J. Environ. Monit.* (**Chapter 4.2**)

Papers on related subjects:

R.J. Law, J. Klungsøyr, P. Roose and W. De Waal, QUASIMEME Workshop III: Summary of the Seminar and Poster Sessions. *Mar. Poll. Bull.* 29 (1994) 217-221.

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L. Holsbeek, C. Joiris, V. Debacker, B.A. Ishaque, J. Nelissen, P. Roose, J. Bouquegneau and M. Addink, Stable pollutants – heavy metals, organochlorines and polycyclic aromatic hydrocarbons – in sperm whales stranded in the southern North Sea during the 1994/1995 Winter. *Mar. Poll. Bull.*, 40 (2000) 1167-1177.

P. Roose, Bioconcentration and hazard assessment of Volatile Organic Compounds (VOCs). *Report of the Marine Chemistry Working Group,* Annex 10, ICES, Copenhagen, Denmark, 2002.

B.J. Versonnen, P. Roose, E.M. Monteyne and C.R. Janssen, Estrogenic and toxic effects of methoxychlor on zebrafish (*Danio rerio*). *Environ. Toxicol. Chem.*, 23 (2004) 2194-201.

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