

ANNEX 8: VOLATILE ORGANIC COMPOUNDS IN VARIOUS MARINE ORGANISMS FROM THE SOUTHERN NORTH SEA

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ABSTRACT

The concentration levels 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, concentration levels 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, C₂-substituted benzenes, CHCs and benzene plus toluene. This division could be related to different types of sources. Finally, it was shown that organisms can be used to monitor the presence of VOCs in the marine environment and the observed concentration levels were compared with proposed safety levels.

Keywords: VOCs, marine organisms, southern North Sea

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 (Baker and Eisenreich, 1990; Clark *et al.*, 1990; Södergren *et al.*, 1990; Mackay and Paterson, 1991; Walker and Livingstone, 1992). 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 (Sweet and Vermette, 1992; Bellar *et al.*, 1974; Anderson *et al.*, 1991; Jungclaus *et al.*, 1978). 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 (Howard, 1990). In Belgium, the emissions of the chlorinated hydrocarbons (CHCs) chloroform, 1,1,1-trichloroethane, tri- and tetrachloroethylene, 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) (GESAMP, 1990) 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 (Ministerial Declaration of the Third International Conference on the Protection of the North Sea, 1990; Ministerial Declaration of the Fourth International Conference on the Protection of the North Sea, 1995) and are recognised as compounds that present an environmental problem by the Norwegian Pollution Control Authority (Dons and Beck, 1994). 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 (Ministerial Declaration of the Third International Conference on the Protection of the North Sea, 1990; Ministerial Declaration of the Fourth International Conference on the Protection of the North Sea, 1995).

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 (Oslo and Paris Commissions, 1990). Levels reported in the literature are therefore mostly the result of once-only surveys. Pearson and McConnell (1975) 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⁻¹ wet weight. Since that time, similar concentrations have been reported for different organisms from various marine locations (Pearson and McConnell, 1975; Dickson and Riley, 1976; Ferrario *et al.*, 1985; Yasuhara and Morita, 1987; Gotoh *et al.*, 1992). An overview of the reported concentrations for the different trophic levels is given in Table A8.1. Recent findings agree with these earlier observations (Roose and Brinkman, 1998). 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 (North Sea Task Force, 1993).

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 (van Leeuwen *et al.*, 1992). 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 (Wong *et al.*, 1992). Also, trichloroethylene has been shown to produce tumours in rodents and is a suspected human carcinogen (WHO Working Group, 1985). 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 (Wong *et al.*, 1992). Benzene is also a known leukemic agent in humans (Sittig, 1980; Kalf *et al.*, 1987; WHO Working Group, 1993). Finally, benzene and its metabolites inhibit both nuclear and mitochondrial replication and transcription in mice (Kalf *et al.*, 1987). Moreover, during metabolism both benzene and its metabolites are converted to reactive species that covalently bind to macromolecules like DNA, RNA and proteins (Kalf *et al.*, 1987). 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.* (1992) 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 Matthiessen *et al.* (1993) 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 concentration levels 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.

MATERIALS AND METHODS

Sampling

Samples were taken on board the Belgian oceanographic vessel “*Belgica*” at six different locations (Figure A8.1) 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 (Oslo and Paris Commissions, 1990). 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.

Analytical methodology

A detailed description of the analytical methodology is given elsewhere (Roose and Brinkman, 1998). 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, Cincinnati, 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 Vocab

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⁻¹. 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 (Figure A8.2). 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⁻¹ wet weight (1,2-dichloroethane, 1,1-dichloroethane and tetrachloromethane) and 0.2 ng g⁻¹ wet weight (chloroform) depending on the background levels and the amount of sample (Roose and Brinkman, 1998).

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) (Roose and Brinkman, 1998). 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) was 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 (Motulsky, 1995). 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.

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 (Figure A8.3). The 75 percentiles (75P) of all CHCs, with the exception of chloroform, were below 2 ng g⁻¹ wet weight and the medians were below 1 ng g⁻¹ for all the species and tissues that were analysed. Tetrachloromethane and 1,1-dichloroethane could not be detected (< 0.005 ng g⁻¹ wet weight) in a significant number of samples. For the other CHCs, except chloroform, the 75Ps varied between 0.02 ng g⁻¹ for trichloroethylene and 1.5 ng g⁻¹ 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⁻¹ wet weight. Pearson and McConnell (1975), Dickson and Riley (1976), Ferrario *et al.* (1985) and Gotoh *et al.* (1992) 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 (Pearson and McConnell, 1975). 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.* (1998). 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 (Howard, 1990). 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, concentration levels found in this study are similar to those reported in the literature (Table A8.1). 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 (WHO Working Group, 1987). 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 (WHO Working Group, 1984). 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⁻¹ for benzene in shrimp and 28 ng g⁻¹ for toluene in *Macrura stultorum*, and the median values varied between 0.08 ng g⁻¹ wet weight for benzene and 22 ng g⁻¹ for toluene for the same species, respectively (Figure A8.3). That is, the concentrations were at least an order of magnitude higher than those of the CHCs. The concentrations of the C₂-substituted benzenes in Figure A8.3 show closely related patterns. This suggests that they are correlated, i.e., have a common source (see below). No such similarity was found for benzene and toluene, which have concentrations that are sometimes higher, and sometimes lower, than those of the C₂-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 metabolism, because MAHs are known to be readily metabolised in organisms (ECETOC Working Group, 1986; Anon., 1988; Slooff, 1988; Crookes *et al.*, 1993). Furthermore, elimination of MAHs from organisms appears to be fairly rapid once exposure has ceased (ECETOC Working Group, 1986; Anon., 1988; Slooff, 1988; Crookes *et al.*, 1993).

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.* (1985) reported concentrations of benzene, toluene and ethylbenzene in clams (*Rangia cuneata*) and oysters (*Crassostrea virginica*) from the Lake Ponchartrain estuary (USA). Benzene exhibited the highest concentrations in both clam (260 ng g⁻¹ wet weight) and oyster (220 ng g⁻¹ wet weight). The concentrations of toluene and ethylbenzene were significantly lower (maximum, 18 ng g⁻¹ 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 (1987) 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⁻¹ wet weight for benzene to 0.25 µg g⁻¹ 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.

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 (Figure A8.4) 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 (Roose and Brinkman, 1998), 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.

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 (Figure A8.5). The clustering is most probably the result of different concentration levels, 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 C₂-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*- and *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 C₂-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 for 40 %, 16 %, and 12 %, respectively, of the total variance of all samples. From the factor loading plot after varimax rotation (Figure A8.6) it was clear that Factor 1 was mainly determined by the C₂-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 concentrations of, especially, ethylbenzene and the xylenes and, to a lesser extent, benzene and toluene. This first principal component further allows making a distinction between MAHs and CHCs, since the latter hardly contribute to this factor.

When the factor scores of all samples are considered (Figure A8.7), no distinct clusters of samples could be distinguished on the basis of species type, tissue type, 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 A8.7 (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).

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 use of fossil fuels in winter) and biological parameters (such as preferential accumulation in certain tissues, metabolism, 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 C₂-substituted benzenes are closely related to each other. The largest emission source of ethylbenzene and the xylenes is gasoline (ECETOC Working Group, 1986; Howard, 1989, 1990; Crookes *et al.*, 1993) 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.* (1998). 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 (Howard, 1990). Finally, the lack of differences between the various sampling stations allows us 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 all year round and that the sources are essentially constant in nature.

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.* (1992). 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 a passive partitioning of a chemical compound between the aqueous phase and a lipid or a lipid-like organic phase (van Leeuwen *et al.*, 1992). 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):

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

This coefficient is supposed to be an intrinsic property of the chemical and can, as a result, be related to its octanol-water partition coefficient, K_{ow}. Neely *et al.* (1974) and, subsequently, several other authors (Isnard and Lambert, 1988;

Connell and Hawker, 1988; Banerjee and Baughman, 1991) demonstrated that BCF and K_{ow} are linearly related according to:

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

The data obtained during the present study were compared with the average water concentrations reported by Dewulf *et al.* (1998) 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.* (1974) are included in the picture (Figure A8.8). 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.* (1974) (Figure A8.8). 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. A possible explanation for this could be the use of nominal instead of actual concentrations. BCFs reported in the literature are often the results of laboratory experiments in open systems and nominal concentrations can easily be too high due to the high volatility of the compounds of interest (Crookes *et al.*, 1993). 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.* (1992) 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. 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:

$$HC5_w = C_w \times (1 + 1.85 \times 10^{-6} K_{ow}) [4]$$

where $HC5_w$ is the total concentration in the water phase, including suspended matter. The internal tissue concentration, ITC or $HC5_{org}$, for the organisms was calculated from

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

where a lipid content of about 5 % by weight in the organism is assumed. Table A8.2 compares the calculated $HC5_{org}$ values and the average concentrations in the different organisms and tissues. The results show that the $HC5_{org}$ for the MAHs and CHCs was not exceeded, in any case. 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 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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Figure A8.1. Sampling stations along the Belgian and Dutch continental shelves.

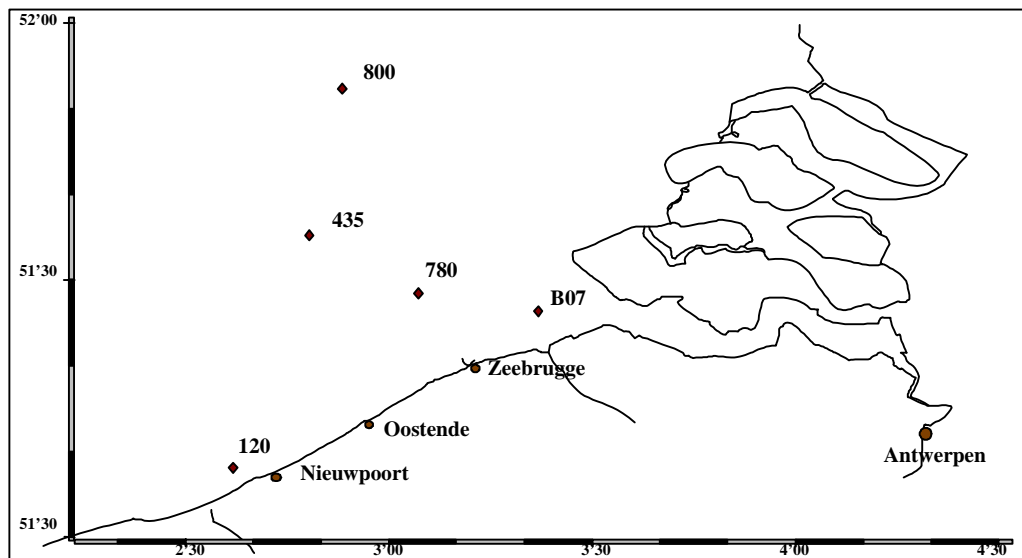


Figure A8.2. Representative total ion chromatogram of VOCs in a shrimp (*Crangon crangon*) sample with the mass spectrum of toluene (insert a) and the selected ion chromatogram of tetrachloroethylene (insert b).

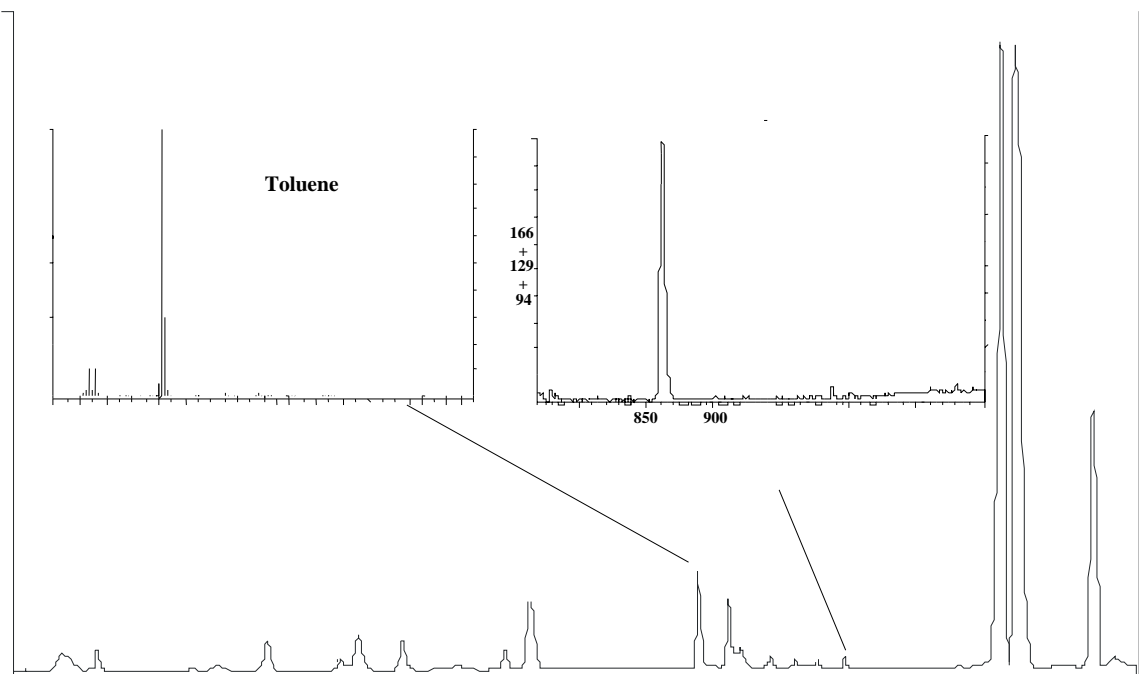


Figure A8.3. Concentrations in ng g-1 wet weight of CHCs (top) and MAHs (bottom) in Crangon crangon (Ccran), Limanda limanda liver (LimaLi), Limanda limanda muscle (Lima Mu), Mactra stultorum (Mac), Merlangius merlangus liver (MerlLi), Merlangius merlangus muscle (MerlMu), Mya truncata (Mya), Spisula subtruncata (Spis) and Venerupis pullastra (Vene).

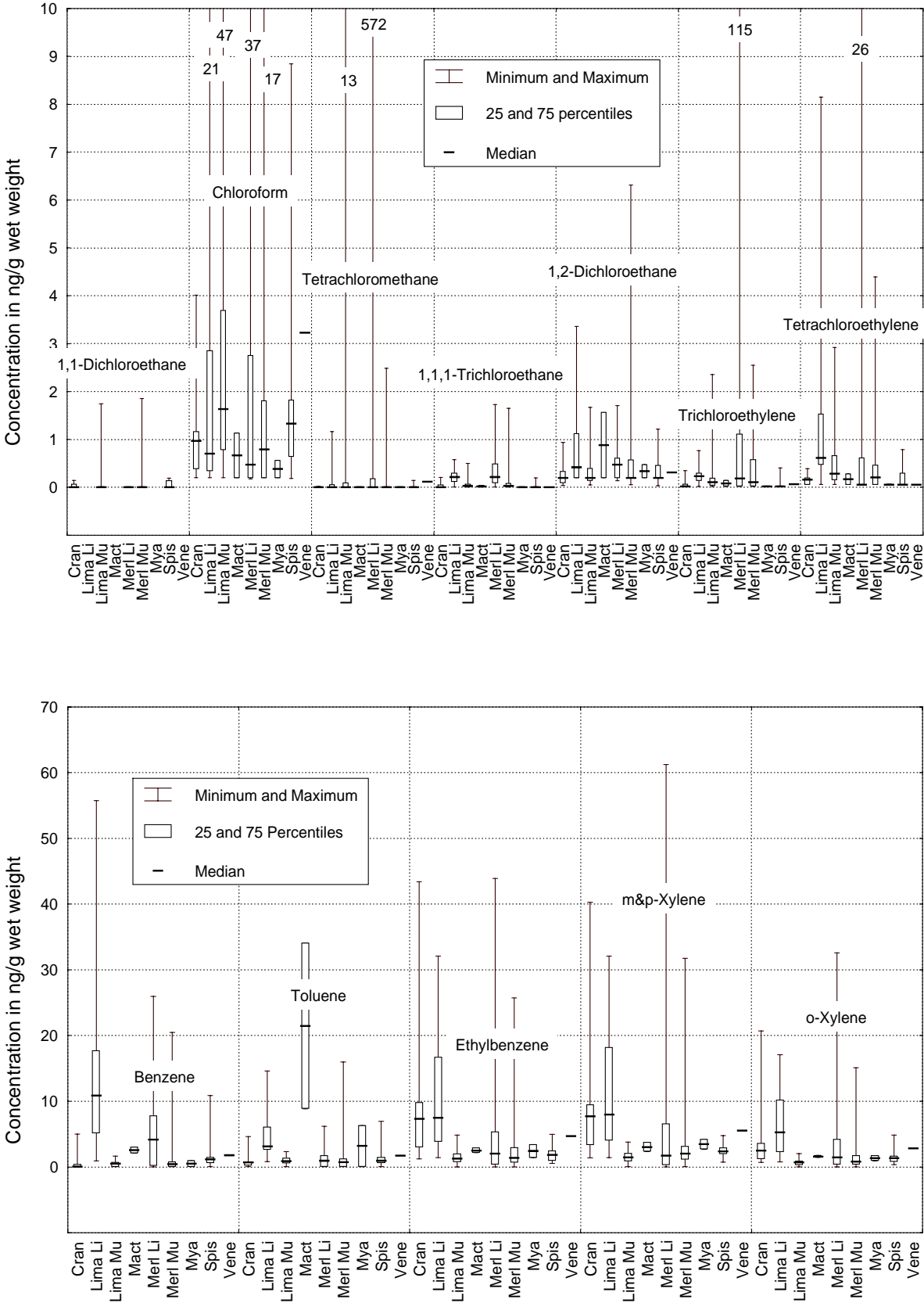


Figure A8.4. Normal probability plot for the trichloroethylene data of the present study.

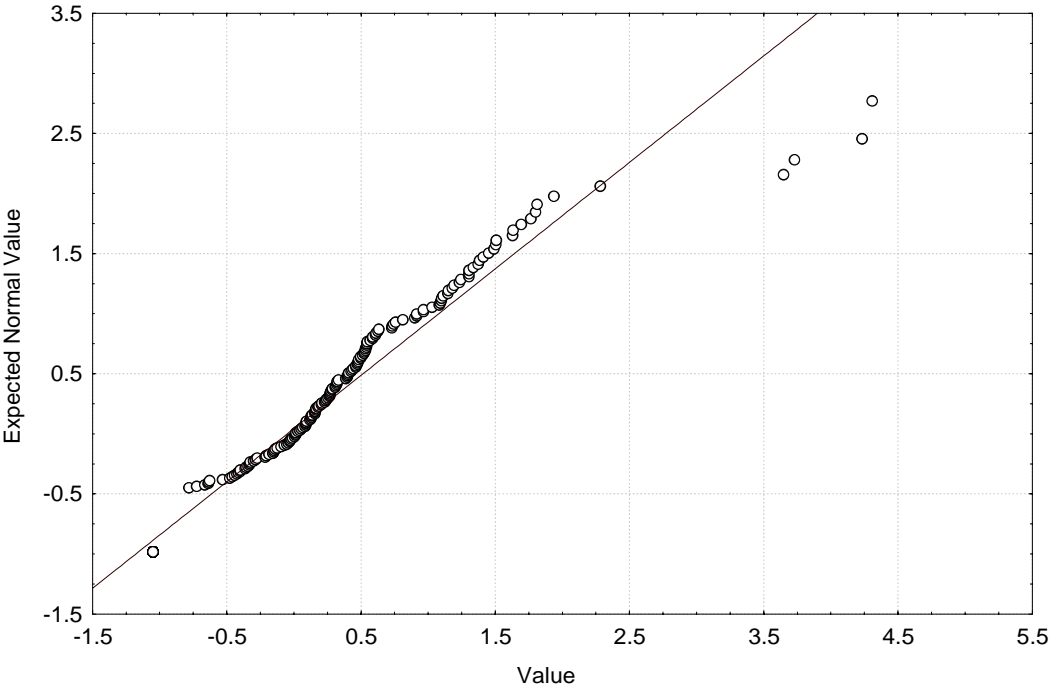


Figure A8.5. Horizontal hierarchical tree-plot representing the variable-wise cluster analysis of VOCs in marine organisms. (BENZ = benzene, TOL = toluene, EBEN = ethylbenzene, MPBEN = *m&p*-xylene, OBEN = *o*-xylene, CHCL3 = chloroform, CCL4 = tetrachloromethane, DCE12 = 1,2-dichloroethane, TCE = trichloroethylene, TECE = tetrachloroethylene and TRCE = trichloroethylene).

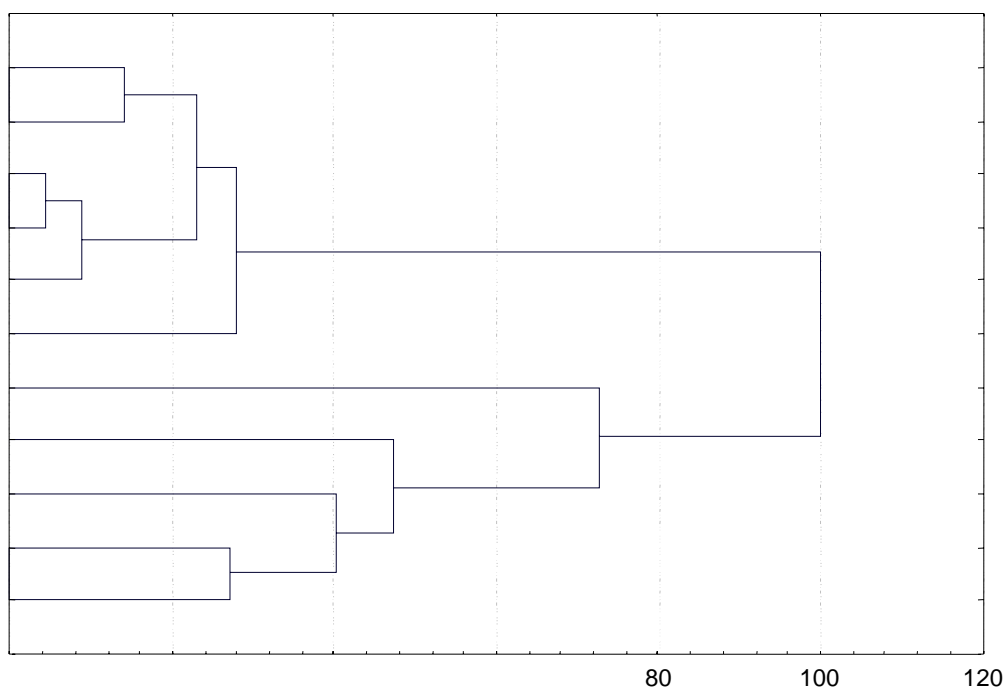
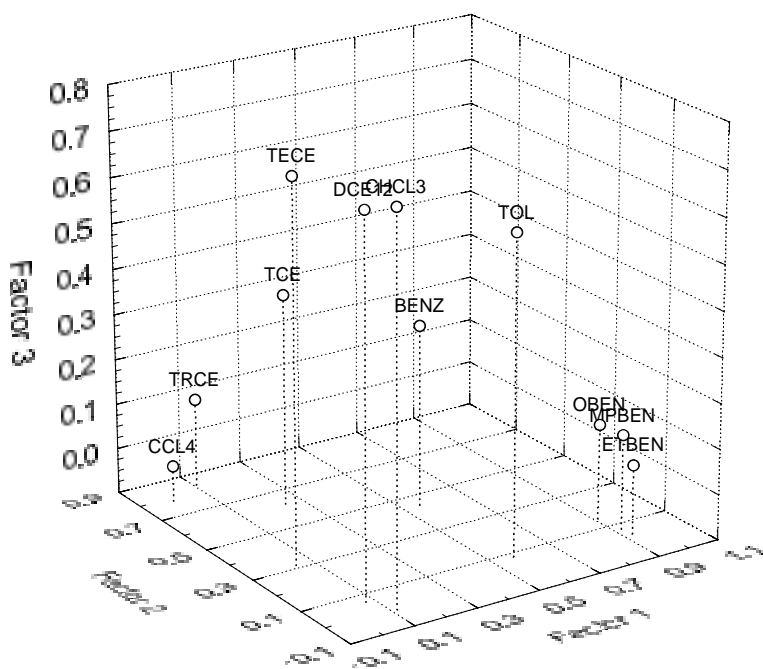


Figure A8.6. Three-dimensional plot of the factor loadings for the different VOCs after varimax rotation.



Scatter plot showing Factor 1 (X-axis) versus Factor 2 (Y-axis). The plot displays numerous data points, each labeled with a case number. The X-axis ranges from -4 to 4, and the Y-axis ranges from -3 to 6. A cluster of cases (111, 112, 113, 114) is highlighted with an oval, indicating a group of cases with similar factor scores. Other cases are scattered across the plot, with some outliers like Case 108 and Case 100.

Figure A8.8. 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.

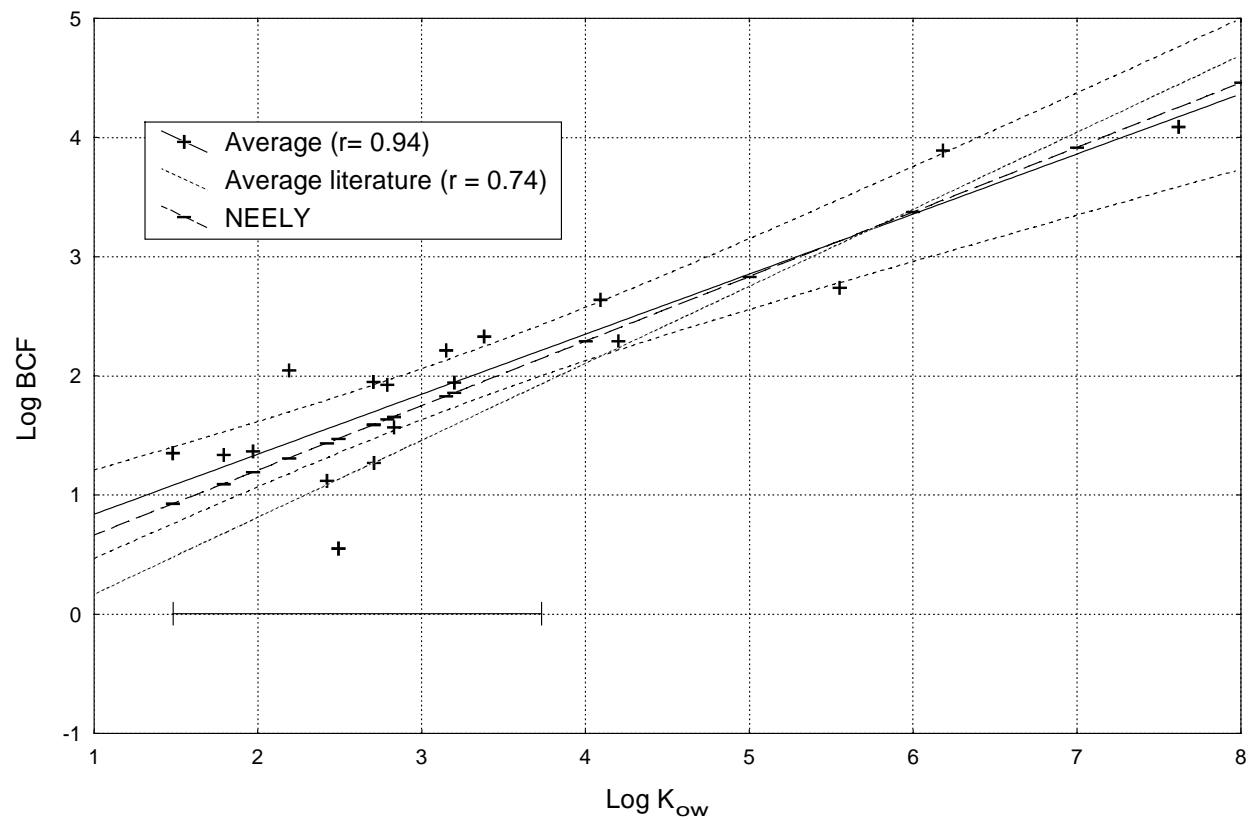


Table A8.1. Concentrations in ng g⁻¹ wet weight of VOCs in various marine organisms (10,16–21).

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
	14.1 MAHs					
		BENZ	TOL	EBEN	MPBEN	OBEN
Shellfish		220–7000	3.4–18	0.8–250	100–360	520
Fish		700–1000				

CHCl₃ = chloroform, CCl₄ = 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.

Table A8.2. Comparison of the average tissue concentrations in pg g⁻¹ of the present data set and the proposed safety level (HC5).

Compound	<i>Crangon crangon</i>	<i>Mactra stultorum</i>	<i>Mya truncata</i>	<i>Spisula species</i>	<i>Limanda limanda</i> liver	<i>Limanda limanda</i> muscle tissue	<i>Merlangius merlangus</i> liver	<i>Merlangius merlangus</i> muscle tissue	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
<i>m&p</i> -Xylene	9700	3000	3500	2500	11000	1500	6300	3200	6.4×10^6
<i>o</i> -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
Tetrachloromethane	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