Impulse Programme Marine Sciences 1992-1996

Project n° 4

Intercompartment distribution of monocyclic aromatic hydrocarbons and C_1-C_2 organochlorines in the North Sea environment

Technical Summary

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TECHNICAL SUMMARY

1. Literature study

A thorough literature study was carried out. It was focussed on the available analytical methods for the measurement of the target compounds in the different environmental compartments, on their physico-chemical behaviour in the marine environment and on the reported concentration levels of the compounds in the marine environment.

AIR. The sampling and analysis in the air compartment is frequently done by sorbent preconcentration and subsequent thermal desorption or solvent desorption. Next, sampling by means of canisters and cryogenic preconcentration is reported. The compounds are analysed by gas chromatography. The limits of detection depend on the applied method and are reported to be in the parts per quadrillon to parts per billion range. An extended overview of the results of measurement campaigns in the marine environment is discussed.

WATER. Sampling techniques for the water compartment, such as the use of Transparent Plastic Nansen (TPN) samplers, Go-Flo bottles and Niskin sampling bottles, are discussed. The different techniques to store the samples, e.g. cooling of the samples and the addition of anti-microbial agents, are summarized. Frequently used preconcentration methods are the solvent extraction technique (especially for chlorinated hydrocarbons) and the purge and trap method. Also new techniques (Solid Phase Micro-Extraction (SPME), Membrane Introduction Mass Spectrometry (MIMS), Membrane Extraction Micro-Trap preconcentration (MEMT)), which may have applications in the marine research, are mentioned. After preconcentration, the compounds are separated by gas chromatography. Depending on the technique, limits of detection in the parts per trillion to parts per billion range are reported. Measurements in the marine environment showed higher concentration levels in estuaria and bays (parts per billion to parts per trillion) than at open sea locations (parts per quadrillion to parts per trillion).

Further on, mainly the anthropogenic sources of the target compounds are summarized, indicating that the compounds can be brought into the marine environment in two ways, i.e. by atmospheric input or via the water compartment. For the behaviour and the transport of the target compounds in

the marine environment, it is clear from the literature study that the air/water exchange is an important exchange mechanism.

SEDIMENT. Sampling techniques, such as the Van Veen sampling method, are discussed, next to methods in order to store the samples in a suitable way (e.g. cooling, freezing, ...). For the analysis of the samples, several preconcentration method are reported (purge and trap preconcentration, vacuum extraction technique, static headspace technique, ...). The separation is carried out by gas chromatography. Analytical aspects, such as the recovery and the limits of detection (parts per trillion to parts per billion) are discussed. Measurements in estuarine sediments showed concentrations in the range of parts per trillion to parts per billion. The partitioning over the different fractions within the sediment is discussed, showing that the organic fraction is the main fraction with respect to the uptake of the target compounds in the sediment.

BIOTA. The literature study dealt first of all with the sampling and storage techniques for the analysis of VOCs in marine biota. Little is found in literature concerning the sampling of biota but it can be assumed that traditional fishing techniques such as beam trawling are used. Several authors reported storage techniques that are mainly aimed at diminishing or eliminating losses of or contamination due to the analytes. Reported techniques, in that respect, were storage on dry ice and in the freezer. The next part of the literature study was dedicated to the discussion of analytical techniques. Few authors described analysis methods for the determination of VOCs in marine biota. The most important pre-concentration techniques that were applied, were the purge and trap technique (P&T), vacuum-distillation and steam-distillation. The mixtures that were thus obtained were always separated and analysed with gas chromatography systems. The literature study further discusses the main analytical qualities of the various techniques such as recovery, reproducibility and detection limits (LODs). Next, concentration levels and biological effects were discussed. The concentration levels reported in the rather limited number of publications, indicated that the levels are generally in the low ppb (parts pro billion) to low ppm range (parts pro million). Finally, little was also found regarding the possible effects on marine organisms. The target compounds were reported to exhibit a general narcotic effect and it can be assumed that the early development stages are the most sensitive. A limited potential to bioconcentrate was further reported.

2. Evaluation and application of the analytical techniques

The available techniques for the analysis of the target compounds in the compartments air, water, sediment and biota were evaluated. Based on this evaluation they were further developed and applied in the monitoring campaigns.

AIR. Considering the evaluation of the sorbent sampling technique and the canister sampling method, the sorbent sampling technique, using Carbopack B and Carbosieve SIII sorbent materials, was preferred. For the analysis of the sampling sorbents, it was proven that the analysis in an on-line system did not show the required analytical quality in terms of limits of detection, contamination levels, accuracy and reproducibility. Subsequently it was proven that a thermal desorption in an off-line system, followed by an analysis in the on-line system, provided the appropriate analytical performance. The analytical quality was assessed by means of the assessment methods proposed by the QUASIMEME working group. For the calibration of the samples, the dynamic vapour pressure method and the closed two phase system calibration method (Dewulf J., Ponnet D. and Van Langenhove H., International Journal of Environmental Organic Chemistry, 62, 289-301, 1996) were evaluated. This latter technique was developed in the laboratory and applied in order to calibrate the monitoring campaign results.

WATER. In a first approach, an on-line purge and trap-GC-MS system was evaluated for the analysis of the target compounds in the water samples. The on-line system showed high background levels and a limited reproducibility. Subsequently a method, based on an off-line purge and trap pre-concentration step and an on-line thermal desorption-GC-MS analysis, was developed. This method showed the required analytical performances with respect to limits of detection, contamination levels, accuracy and reproducibility (Dewulf J. and Van Langenhove H., International Journal of Environmental Analytical Chemistry, 61, 35-46, 1995). The technique was assessed by means of the quality assessment methods proposed by the QUASIMEME working group.

SEDIMENT. The coupling of a commercial P&T apparatus to the GC-MS (gas chromatograph-mass-spectrometer) formed the basis for the development of a suitable analytical technique for the determination of the target compounds in marine sediments. The choice was based on the methods reported in literature, of which the P&T technique seemed the most promising. Initial

test were performed, using the commercial set-up on water and sediment samples. However, a number of problems became immediately evident. The first and biggest problem was the presence of relatively high background concentrations of the target compounds. Secondly, severely distorted mass-spectra were obtained as a result of excess water in the purge gas. This water vapor was formed during purging of the sediment samples at elevated temperatures. The latter was imperative to quantitatively volatilize the VOCs out of the sediment samples. The next series of tests was therefore dedicated to eliminate both the water vapor and the background concentrations. To this purpose an off-line method was developed and used. The presence of water vapor could be largely eliminated. However, the background concentrations were, all efforts in vain, never entirely eliminated. The detection limits of the method were therefore based on the background concentrations and the off-line method was further validated. In spite of the shortcomings, the method proved to be of sufficient quality to initiate a first series of analyses on sediment samples. Later on, an on-line method was developed for the analysis of the target compounds in marine biota. The latter was then evaluated and validated for the analysis of marine sediments. The on-line method proved superior to the off-line method and was as a result used, from that point onward, for the determination of the target compounds in marine sediments.

BIOTA. The development of a suitable analytical method for the analysis of VOCs in marine organisms proceeded initially on the basis of the off-line method developed for the sediments (see earlier). In the first instance, sample handling and pre-treatment was studied. This included determining whether homogenization was needed and under what conditions e.g. type of homogeniser and cooling of the sample during homogenization. Even during the initial experiments, two main problems could be identified. As for the sediments, the presence of background concentrations hindered the analysis. More importantly, however, the biological samples foamed excessively under certain conditions of temperature and purge-gas flows. The problem of sample foaming was therefore extensively studied and finally foaming was controlled by using a mechanical barrier (glass wool) and low purge-gas flows. More recently the addition of n-octanol as an anti-foaming agent was studied with considerable success. The tests mentioned above culminated in the development and validation of an off-line technique for the determination of the target compounds in marine organisms. This method was then compared

with an identical on-line set-up which proved superior in mainly in terms of repeatability. From then on, only the on-line method was used for the determination of VOCs in biological material.

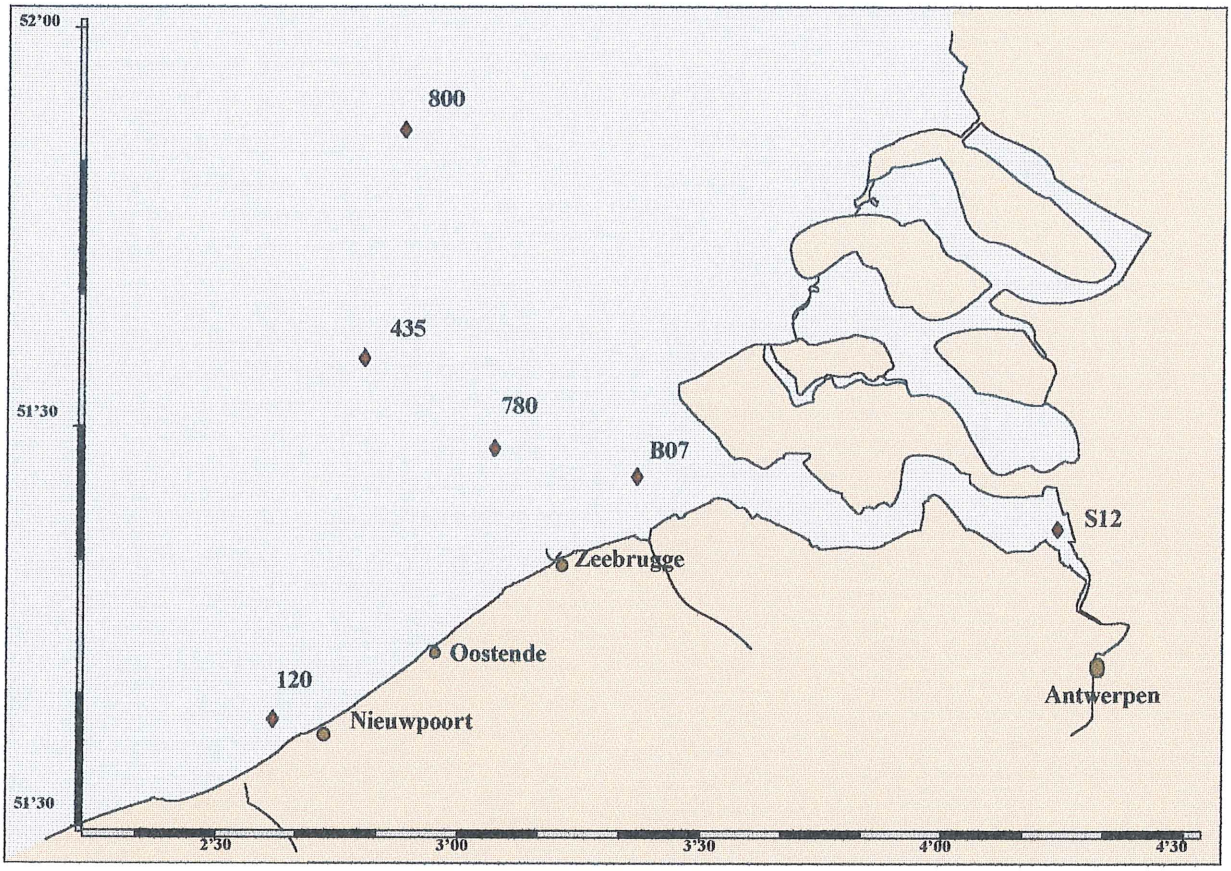

Figure 1. Map of the sampling locations according to the programme and one additional reference sampling location (location 800) in the Scheldt estuary and on the Belgian Continental Shelf Sea

3. Monitoring of the target compounds in the marine environment

The six sampling locations provided in the programme are illustrated in Figure 1. Additionally to the programme, one reference location is indicated (location 800). Four intensive sampling campaigns

for the water compartment were carried out in the Scheldt estuary, additional to the programme. The sampling locations of these monitoring campaigns are presented in Figure 2.

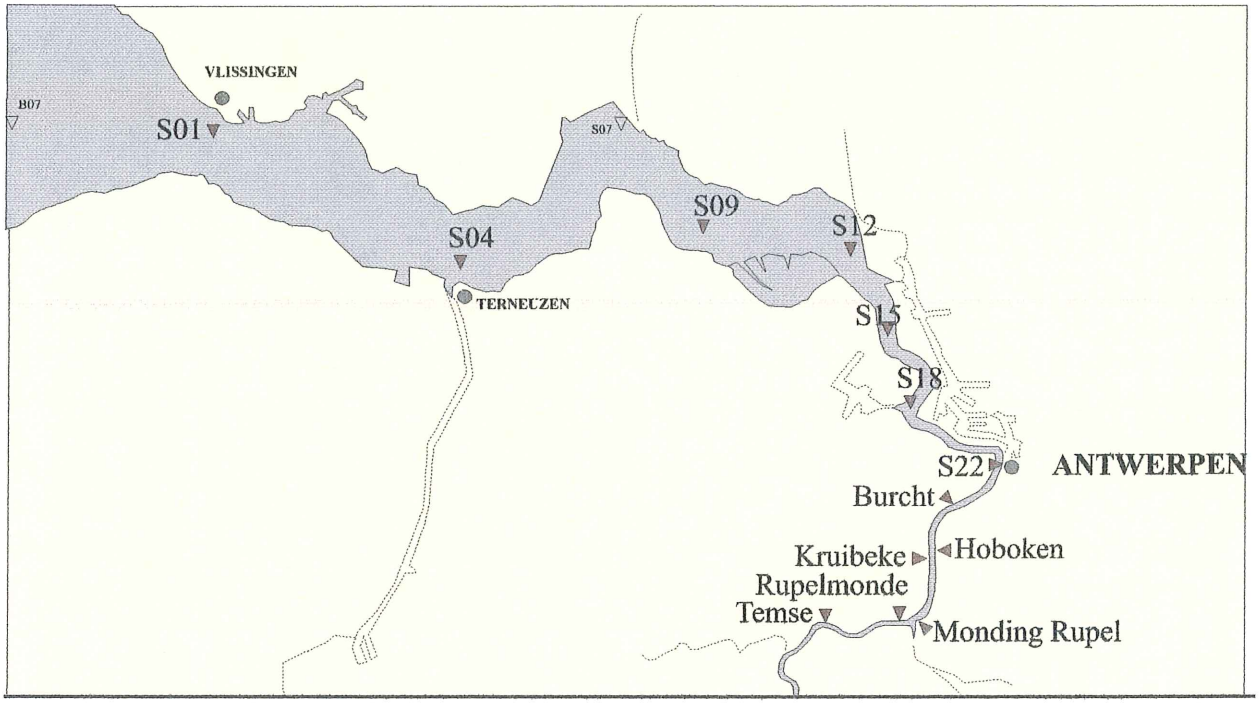

Figure 2: Sampling stations along the Scheldt.

AIR. The variability of the atmospheric concentrations of the target compounds was examined by a consecutive sampling during a 2.5 hours period on one location. Most target compounds showed variances in the concentrations between 16 and 33% (n=5). Exceptions were chloroform and 1,1-dichloroethane (41%). Further on, the C_2-substituted monocyclic aromatic hydrocarbons proved to have variances in the concentration levels between 44 and 76%.

The programme provided a three years monitoring campaign with four sampling campains a year (1993, 1994 and 1995). In the programme, two sampling sites in the Scheldt estuary (at the mouth and at Doel), two locations near the coast (4-15km) and two locations far from the coastline (35-40km) were mentioned. An additional location was sampled as a reference (60km off the coast). Air

sampling could not be carried out in the first year and in the first campaign of the second year due to the development of the analytical technique at that time. Moreover, the second campaign of the second year was not carried out because no campaign was provided by the research vessel the *Belgica* during that period. Finally, a number of locations were not sampled in the fourth campaign of the second year due to bad weather conditions.

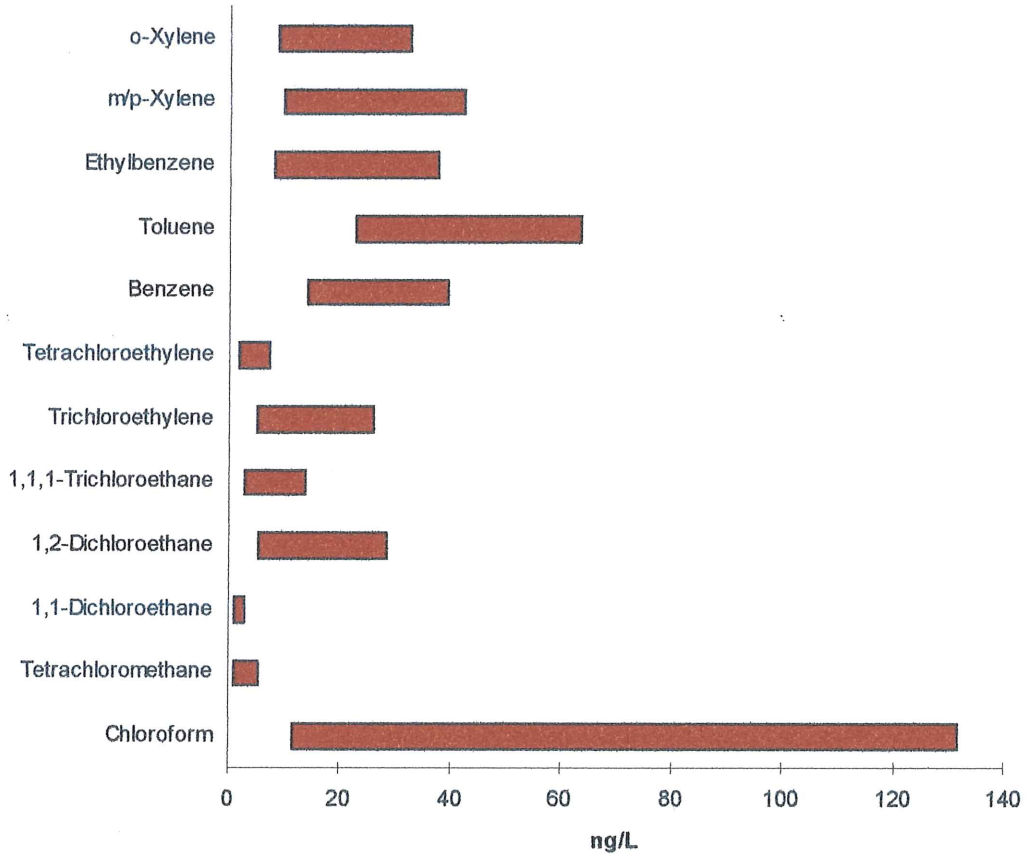

Figure 3. 25 to 75 percentile ranges of the measurements of atmospheric concentrations of the target compounds

Figure 3 represents the 25 to 75 percentile ranges of the target compounds for the complete dataset (n=38). Within the group of the chlorinated compounds it can be seen that 1,1-dichloroethane shows concentrations below 10ng.m^{-3}, whereas chloroform and 1,2-dichloroethane concentrations vary between 10 and 100ng.m^{-3}. The concentrations of trichloroethylene and tetrachloroethylene are

between 100 and 500ng.m^{-3}, those of 1,1,1-trichloroethane and tetrachloromethane between 500 and 1000ng.m^{-3}. The concentrations of the individual monocyclic aromatic hydrocarbons are in the 500 to 1500ng.m^{-3} range, excepted those of toluene (up to 3800ng.m^{-3}).
Several statistical tests were applied on the dataset (n=38). Factor analysis did not indicate that one single sampling location or a set of sampling locations showed significant higher concentrations than one other sampling locations or a set of sampling locations. Cluster analysis and principal component analysis proved that a number of samples of the second campaign of 1995 (May) showed enhanced concentrations of C_2-substituted monocyclic aromatic hydrocarbons. No direct explanation could be found so that further research on this aspect is advised.

WATER. The variability of the concentrations in the water column was examined by consecutive sampling on one sampling location. The compounds showed variances in the concentrations between 6 and 44% (n=5).
The programme provided a three years monitoring campaign with four samplings a year (1993, 1994 and 1995). The locations were two locations in the Scheldt estuary (at the mouth and near Doel), two locations near the coast (4-15km) and two locations far from the coast (35-40km). An additional location was sampled as a reference (60km off the coast). The second campaign of the second year was not carried out since no campaign time of the research vessel the *Belgica* was available for the project. Further on, a number of locations were not sampled in the fourth campaign of the second year due to bad weather conditions.

The concentrations of the individual compounds were in general in the order of 1 to 50ng.L^{-1}. This is presented in Figure 3 by means of the 25 to 75 percentile ranges (n=68). Only chloroform showed a higher 75 percentile level (130ng.L^{-1}). From the statistical tests applied on the complete dataset (cluster analysis, principal component analysis and factor analysis) it was proven that the sampling location in the Scheldt estuary near Doel showed enhanced concentrations for all chlorinated target compounds, except for chloroform. On the other hand the sampling location at the mouth of the estuary was not to be distinguished, except for 1,1,1-trichloroethane, which showed significant higher concentrations at this location than at open sea locations.

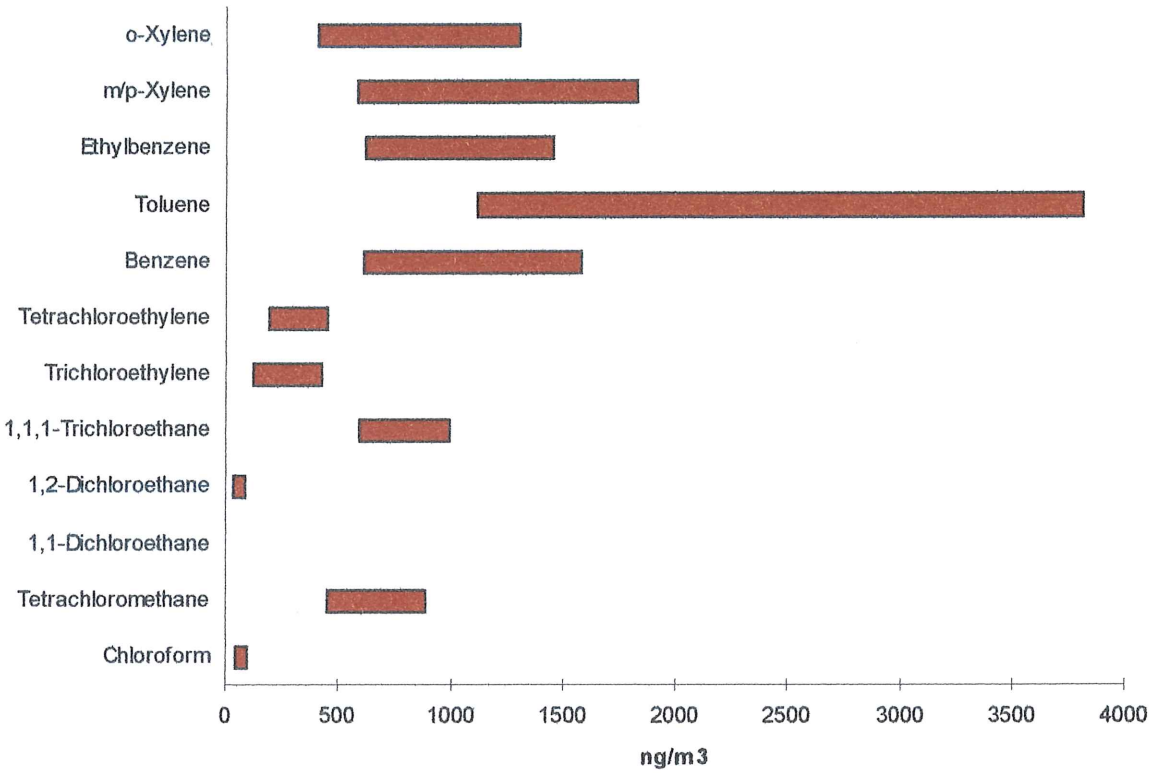

Figure 4. 25 to 75 percentile levels of the measurement results of the target compounds in the water column

Cluster analysis, principal component analysis and factor analysis indicated that the concentrations of chloroform and tetrachloromethane were higher at locations near the coast in the third sampling campaign of the year. According to the literature biogenic sources can be suggested for this observation. Similar as for the air samples, also for the water samples higher concentrations of C_2-substituted monocyclic aromatic hydrocarbons were noticed in the second monitoring campaign of 1995. This observation is a subject to which further attention has to be paid.

Since the sampling location in the Scheldt estuary at Doel showed systematically enhanced concentrations of the chlorinated target compounds, it was concluded to carry out additional sampling campaigns in the Scheldt estuary. In 1995 eight to ten sampling locations were sampled in the Scheldt estuary along the trajectory Antwerp-Vlissingen. These campaigns demonstrated that the chlorinated compounds showed an increasing concentration profile from Vlissingen towards

Antwerp, as is illustrated for trichloroethylene in Figure 4. This profile can be explained by anthropogenic emissions of these compounds in the estuary. It was proven that the decrease of the concentrations towards Vlissingen was not only due to dilution with sea water.

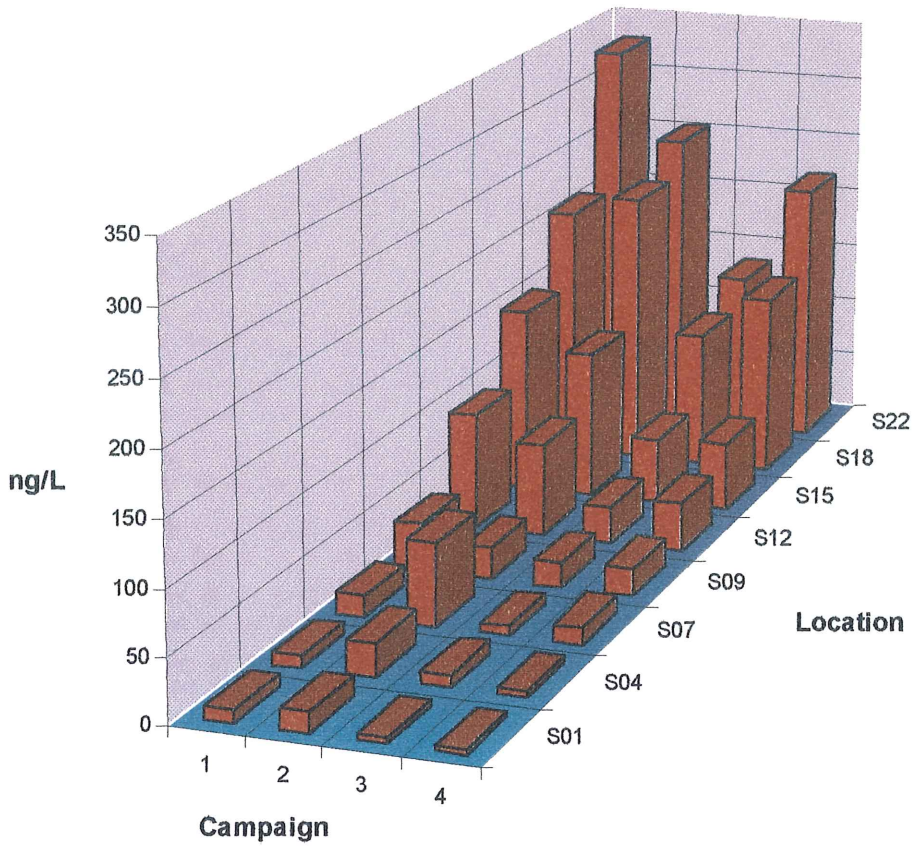

Figure 5. Concentration profiles of trichloroethylene in the Scheldt estuary for the trajectory Antwerp-Vlissingen obtained in the 4 monitoring campaigns in 1995. Location S01 is near Vlissingen whereas location S22 is to be situated in Antwerp.

An additional explanation of the decrease of the concentration is to be found in air/water exchange. The fraction of these compounds which comes into the North Sea via the Scheldt estuary and the fraction exchanged to the atmosphere, can be estimated by modelling. This implies that the concentration profiles must be available (as those obtained in this work). Next, knowledge of the physico-chemical characteristics (See point 4) and emission data are required. This latter element proves to be a limitation for the modelling until now.

SEDIMENT. A first series of analysis led to the conclusion that VOCs concentrations in sediments of the Belgian Continental Shelf (BCS) were hardly ever detectable, despite detection limits of about 0.01 ppb. A possible solution would have been the development of an even more sensitive analysis method, but this was unfortunately nor feasible in the time frame of the project. As a result the attention became mainly focused on the analysis of biological samples. In view of the considerable difficulties that were associated with this analysis and as a result of the slow sample throughput of the method, substantially less samples could be analysed than originally planned. De results were, on the other hand, very positive so absolute priority was given to the analysis of as much biological samples as possible. Only near the end of the program a new series of sediment samples was analysed.

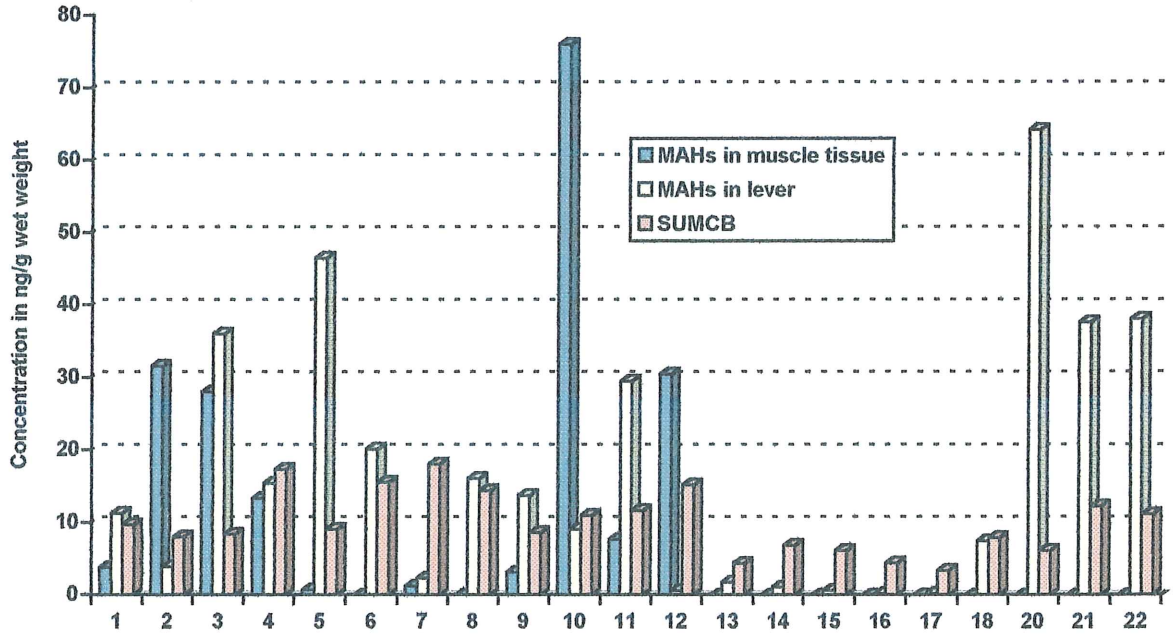

Figure 6: Concentrations of monocyclic aromatic hydrocarbons (MAHs) sum of the PCBs in liver and muscle tissue whiting (*Merlangius merlangus*).

The findings were however the same as before. Only a limited number of detectable concentrations could be discerned. As a result, no seasonal or spatial effects could be demonstrated. It can therefore be stated that concentrations of VOCs in sediment on the BCS

are generally lower than 0.01 ppb. The presence of these compounds in sediments should therefore not be considered as a major problem.

BIOTA. First of all, the interspecies and interspecimen variability was studied for dab (*Limanda limanda*) and whiting (*Merlangius merlangus*). The first remarkable fact was the large variability that could be observed for concentrations in the same tissue type of individuals of the same population (figures 6 and 7).

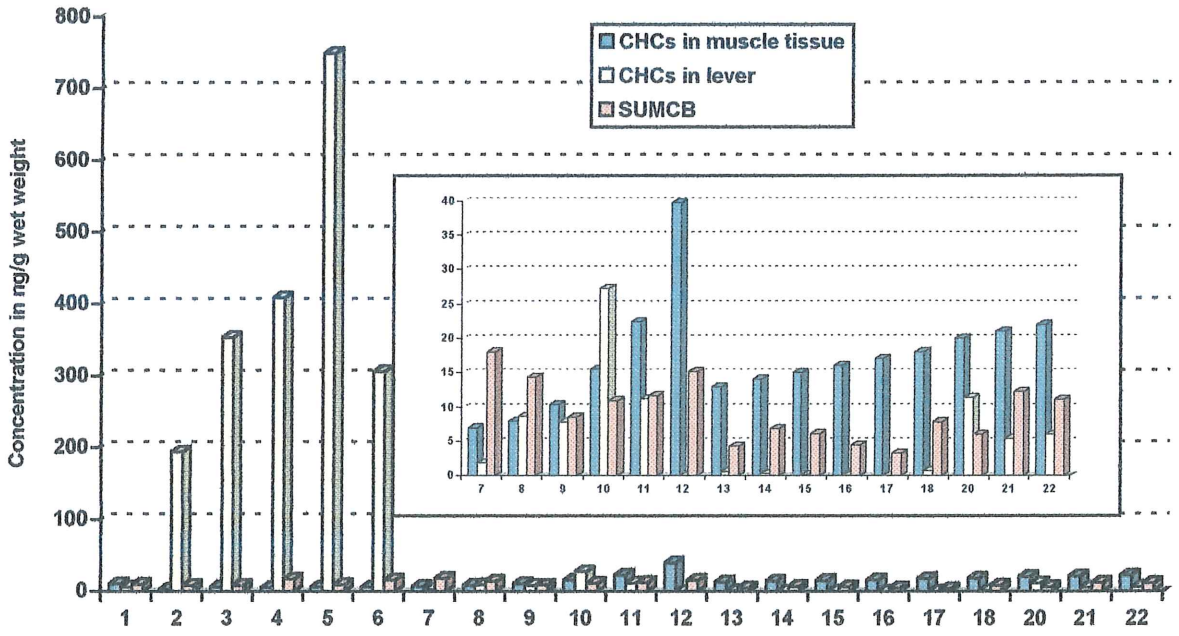

Figure 7: Concentrations of C_1-C_2 organochlorines and the sum of the PCBs (SUMCB) in liver and muscle tissue of whiting (*Merlangius merlangus*).

The distribution of the concentrations was investigated using a variant of the Kolmogorov-Smirnov test and by using normal probability plots. It was concluded that the concentrations of VOCs seemed to be normally distributed in the different tissue types of two species. These results should however be interpreted with the necessary caution as the samples size were relatively small for these particular types of statistical tests. The concentrations of VOCs in

muscle tissue of individual whiting were further correlated with the fat content and compared to the concentrations of PCBs (polychlorinated biphenyls) and OCPs (organochlorine pesticides). For both fish species and tissue types the concentrations were also correlated with the length of the fish.

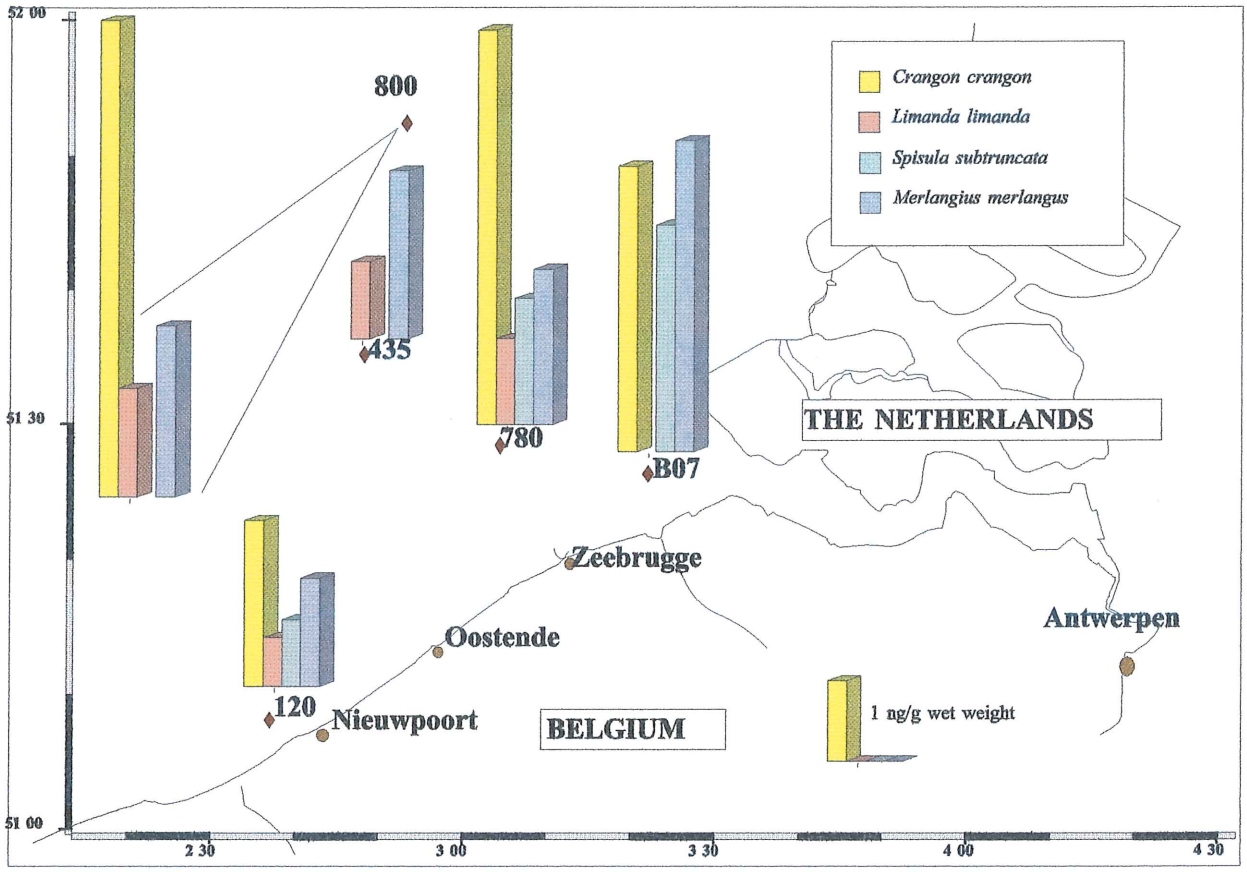

Figure 9: Comparison of the concentrations of monocyclic aromatic hydrocarbons in different species from different location on the Belgian Continental Shelf.

Finally, concentrations in liver and muscle tissue of the same fish were compared and correlated. No distinct relationship could be demonstrated between the fat content and the concentration of the individual VOCs, although the latter was clearly the case for the PCBs. It was therefore concluded that VOCs behave differently and distribute in another fashion in the organism than PCBs. As a result it was further deemed unnecessary to normalise the concentrations of VOCs

on the fat content. The latter is imperative when concentrations of PCBs in different species or at different locations are to be compared. Concentrations in organisms originating from different locations or collected during different seasons were as a result compared on a wet (fresh) weight basis. No significant relationship could further be demonstrated between the concentrations of VOCs and PCBs or OCPs, although the concentration were of the same order of magnitude (figures 7 and 8).

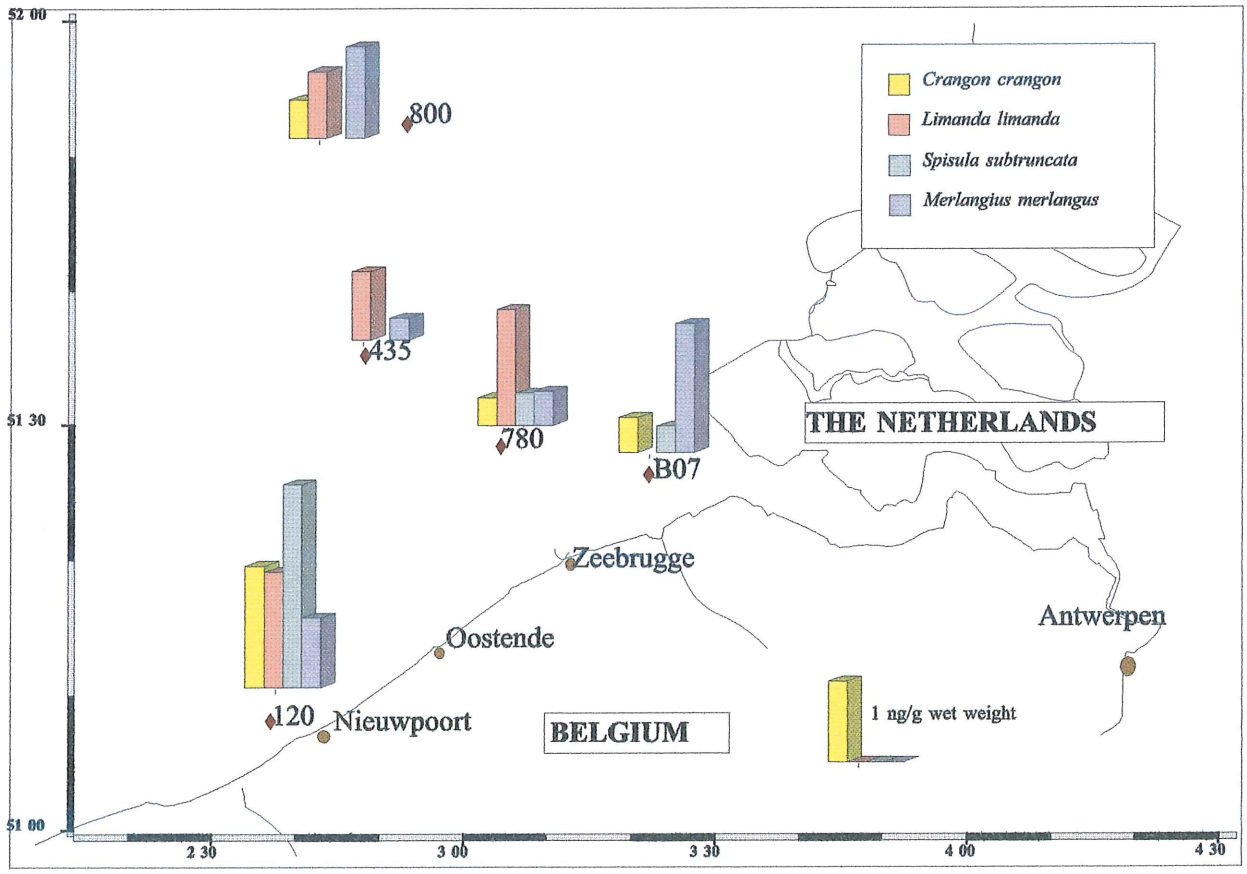

Figure 9: Comparison of the concentrations of C_1-C_2 organochlorines in different species from different locations.

Moreover, no significant relationship could be established between the concentration of the individual VOCs and the length of the organism. It was therefore concluded that no biomagnification occurs in the studied species. The latter is further supported by the fact that the concentrations in organisms of different levels in the food chain are generally not significantly

different (figures 8 and 9). The concentrations in liver and muscle tissue of fish showed also no significant correlation. This lead to the supposition that VOCs bioconcentrate in a different way for different tissues. The observation was also made that concentrations of VOCs in the liver of dab, the main site of metabolisation, were generally higher. It could therefore be assumed that metabolisation is the explanation for these observations. When concentrations in organisms was compared with those in the water column, a bioconcentration factor (BCF) could be calculated. The calculated BCFs were generally a lot higher than those reported in literature. This needs to be further investigated. Finally, no seasonal or spatial differences could be demonstrated for the concentrations in the different organisms (figures 8 and 9). For all practical purposes the BCS could therefore be regarded as one zone.

4. Physico-chemical behaviour of the target compounds

A series of laboratory experiments was carried out in order to investigate the physico-chemical behaviour of the target compounds. Next, a fugacity model was developed to study the behaviour of the compounds in the marine environment. Further on, the developed model was applied on the data available from the monitoring campaigns.

LABORATORY EXPERIMENTS. The physico-chemical behaviour of the target compounds depends on the equilibrium partitioning of the compounds over the compartments air, water and sediment. In a first series of experiments the air/water equilibrium partitioning (Henry's law) was studied intensively (Dewulf J., Drijvers D. and Van Langenhove H., Atmospheric Environment, 29, 232-331, 1995). The EPICs-method (Equilibrium Partitioning in Closed Systems) was further developed and applied. For all 13 target compounds the partitioning was determined as a function of the temperature and the salinity. A bifunctional relationship between these two latter parameters and the air/water equilibrium partitioning constant was established. Further on, it was shown that the temperature and the salt concentration are the only two factors determining the air/water equilibrium distribution in the North Sea environment.

In a second series of experiments the sorption of the target compounds on marine sediment was examined. By means of the miscible displacement technique sediment/water equilibrium partitioning

coefficients were measured (Dewulf J., Dewettinck T., De Visscher A. and Van Langenhove H., Water Research, in press, 1996). A relation with the octanol/water partitioning coefficient was established.

From the equilibrium partitioning coefficients it is found that the compartments air and water are the main compartments with respect to the sink of the target compounds. The sorption on marine (North Sea) sediment is limited, due to the low organic carbon content of the sediment.

DEVELOPMENT AND APPLICATION OF THE FUGACITY MODEL. The fugacity model allows the calculation of the distribution and the transport processes of organic compounds in a defined environment. The development of this model requires the determination of a number of parameters, e.g. the fugacities of the compounds and the fugacity capacities of the compartments. Further on, equilibrium partitioning constants, degradation rates and compartment volumes must be acquired. Finally, transfer processes between the compartments have to be modelled, integrating diffusive and non-diffusive processes. A model was developed for the air/water/sediment/biota-system in the North Sea environment.

The results of the monitoring campaigns allowed the application of the developed model on the measurement results for the compartments air and water, obtained simultaneously. The application of the model showed that the diffusive air/water exchange is the main transfer process for the target compounds (when compared to the other processes, such as wet deposition, transfer to higher altitudes, degradation). In Figure 5 the water to air (and air to water) exchange rates are represented by means of the 25 to 75 percentiles (n=38). For all compounds it is noticed that the exchange rates are in the order of 1 to 10g.km^{-2}.day^{-1} (water to air transfer), with some higher rates for chloroform and m/p-xylene. A principal component analysis revealed that all samples from the Scheldt estuary near Doel, were distinguished because of higher water to air transfer rates of the chlorinated target compounds. These higher exchange rates are to be explained by the enhanced water concentrations. Further on, it was demonstrated by the same statistical approach that higher air to water and water to air transfers of C_2-substituted monocyclic aromatic hydrocarbons were found in the campaign of May 1995. No clear explanation can be suggested for this latter observation.

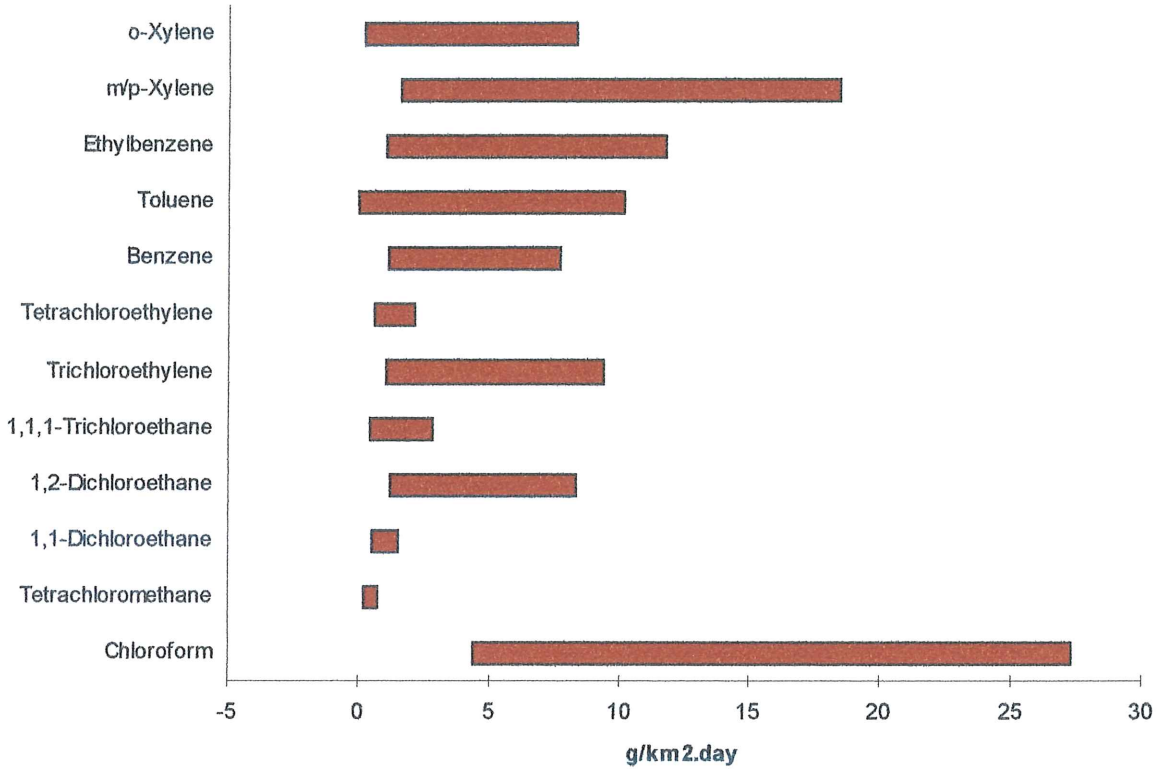

Figure 5. 25 to 75 percentile ranges of the water to air (positive values) and air to water (negative values) transfer rates, calculated from the application of the fugacity model on the simultaneous air and water measurements carried out in the monitoring campaigns.

5. Bioavailability of volatile organic compounds to marine fish (in vitro tests)

The bioavailability of the chlorinated hydrocarbons tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane and the monocyclic aromatic compounds toluene and o-xylene were studied in sole (*Solea solea*). The compounds were introduced in the water tanks via the air input so that exchanges between air, water, sediment and fish could be studied. After 7 days exposure, water, sediment and fish samples were chemically analysed and sublethal effects studied. Special attention was paid to the bioaccumulation from the water since accumulation via feeding was reported to be unimportant.

The chemical results revealed that bioconcentration did not to moderately occur. These values differ thoroughly from the bioconcentration factors calculated from the field data. The latter values may be expected more realistic as the octanol-water partition coefficients of the compounds allow to suggest a certain bioconcentration to occur. A state of non-equilibrium may be the explanation for the low bioconcentration during the *in vitro* experiment resulting in too high water and too low muscle tissue concentrations.

Sublethal effects were studied at the biochemical level by the measurement of ethoxyresorufin O-deethylase, glutathione S-transferase and acetylcholinesterase, swimming behaviour, homeostatic cost (measurement of opercular beats) and the occurrence of external visible diseases and liver lesions. No differences in the biochemical results, swimming behaviour, opercular beats and livers were observed between exposed fish and controls. External visible lesions were solely present after exposure to toluene. All test animals were affected but the lesions were only present on the upper site (site towards the water phase) and not on the lower site. It seems that the sediment layer protected the lower site of the fish, a phenomenon that can only be explained by the absence or unavailability of the administered compounds in the sediment phase. The sediment concentrations of the compounds were indeed totally undetectable. The results also imply that the lesions were caused by direct interactions between water components and the body surface. The lesions occurred on large areas of the upper body surface and were recognised as raised, milky white opaque areas with sometimes haemorrhagic centres. A more evolved stadium was the loss of scales. The lesions did not correspond to know external visible diseases such as epidermal hyperplasias/papillomas or skin ulcers. It may thus be excluded that the main origin is viral or bacterial. The seriousness of the lesions allows to suggest that proliferation may be lethal to the animal. This observation should lead to more long-term studies of the sublethal effects of toluene especially because the compound concentrations in the phases were realistic.