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Determination of volatile organic compounds in marine biota

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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] and Gotoh et al. [4] used solvent extraction techniques and gas chromatography (GC) with ^{63}Ni electron-capture detection (ECD) for analysis. Both groups reported detection limits in the lower ng/g range which were solely due to the high sensitivity and selectivity of ECD for halogenated compounds. It would be impossible to reach similar detection limits using the same techniques for nonhalogenated compounds such as benzene and toluene. Ogata et al. [5] reported the analysis of VOCs using a static headspace technique. 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. However, VOCs are most commonly analysed with dynamic headspace or purge-and-trap (PT) techniques, which are less matrix dependent than static headspace techniques. VOCs are forced out of the tissue by heating in an oven under a flow of nitrogen [6], steam distillation [7–10] or purging with an inert gas [11–13]. The analytes are subsequently trapped prior to analysis using either cryogenic [6–10] or sorbent traps [11–13]. The traps are then desorbed at room temperature [6–10] or by heating at an elevated temperature [11–13] into GC–ECD [6], GC–FID (flame ionisation detection) [13] or GC–MS (mass spectrometry) [7–12].

For this study, we aimed at developing a method that allows the simultaneous determination of halogenated and nonhalogenated 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 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 nonhalogenated 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 setup 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 PT 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 PT apparatus was used both as an on-line PT system and as a desorption unit for off-line PT. After optimisation, the method was applied to the determination of VOCs in two fish species from the Belgian continental shelf.

2. Experimental

2.1. 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, WI, USA) was used as internal standard (I.S.). 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, PA, USA) and used as adsorption traps (1/8 in. O.D.; 1 in.=2.54 cm). 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.

2.2. Apparatus

A microprocessor-controlled PT system, the Tekmar LSC-2000 (Tekmar, Cincinatti, OH, USA), was coupled to a GC–MS system (Finnigan Magnum Ion Trap MS, Finnigan, San Jose, CA, USA) via a heated transfer line terminating in a cryogenic focuser at the GC end. The PT system was provided with a 25-ml fritted sparger and a moisture control module (MCM) as wet trap. The internal lines of the PT 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, IL, 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 and Kunkel (Staufen, Germany) sharing blender and the tissue was further disrupted in a Bransonic (Branson, Danbury, USA) ultrasonic bath.

2.3. Sampling and storage

Fish were caught by the Belgian oceanographic 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 and in the absence of organic solvents. Upon their arrival at the Institute the samples were transported to an airtight freezer located in a separate building and a solvent-free area. Concentrations of VOCs in ambient air were assessed on board the ship and during storage in the laboratory to estimate possible contamination during sampling and storage.

2.4. Analytical procedure

2.4.1. Preparation of blanks

Water specially prepared for the analysis of VOCs (Baker) was used to prepare blanks and standard solutions (see Section 2.4.2 below). The water was pretreated by heating up 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 PT concentration and GC–MS analysis.

2.4.2. 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 mass was recorded. One ml of each of the target compounds was added to the methanol and after each addition the mass was recorded. Finally, the volume was brought to 100 ml and the mass was again recorded to allow correction for possible losses. The procedure enables calculation of the concentration on both a volume and a mass basis. Reporting and using standard solutions on a mass 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 mass basis. Because of the high volatility of the analytes, frequent renewal of standard solutions is recommended. The diluted solutions were continuously 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 a another 10-μl syringe. The water was then injected into a 24-ml sample vial and the sample vial connected to the on-line PT setup, preconcentrated and analysed by GC–MS.

2.5. Sample pretreatment 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 PT 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 PT 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 done on a 60 m×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 p.s.i. (1 p.s.i.=6894.76 Pa) The target compounds were identified on the basis of their retention times and mass spectra and quantified using the total mass of selected ions (Table 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 u and the scan rate 1000 ms. The filament delay was 180 s, and a mass defect of 50 mmass/100 u and a background mass of 45 u were selected.

2.6. 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 solution 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 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 PT related.

Table 1
Retention windows and selected masses of the target compounds

Compound	Retention window (min:s)	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- and *p*-Xylene	12:05–12:25	91, 106
o-Xylene	12:45–12:65	91, 106

2.7. 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].

3. Results and discussion

3.1. System blank and removal of excess water

After the first series of 5-ml blank water samples, consisting of 5-ml water pretreated as described in Section 2.5 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 pretreatment 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 higher levels 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.

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 5 ml blank water 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. The results of these tests are shown in Figs. 1 and 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 (R.S.D.) 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 concentration levels of Fig. 1 should be divided by 4 or, in other words, the headspace concentrations should be below 50 pg/ml. The results of Fig. 2 indeed indicate that the levels in laboratory air are around that level with average concentrations ranging from 10 to 70 pg/ml. Frank et al. [18] reported levels of CHCs in air ranging from 20 to 300 pg/ml at the Atlantic coast of Portugal, while Bianchi and Varney [19] reported levels up to 16 ng/ml over the Southampton Estuary. Levels for MAHs in air at the Bretagne coast (France) ranged from 20 to 600 pg/ml [20] and from 1 to 200 ng/ml 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 meant that the equipment itself was prone to contamination by samples containing high amounts of VOCs. In 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 equip-

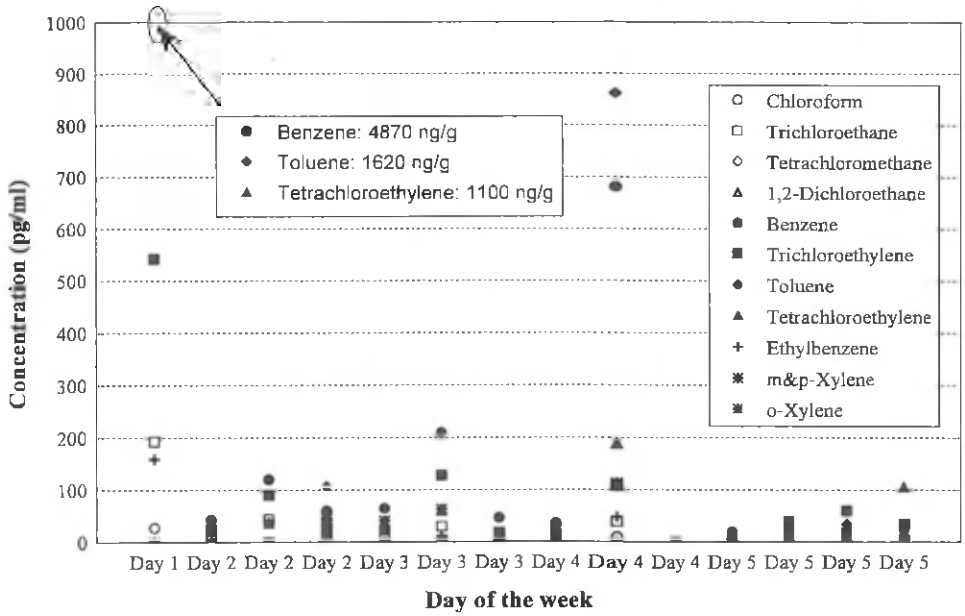

Fig. 1. Analyte concentrations in water blanks recorded over a period of one week.

ment. During these initial tests, it also became evident that the MCM of the PT 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 PT device. Eliminating the water vapour formed during purging was therefore a prerequisite for a proper analysis. To remedy the

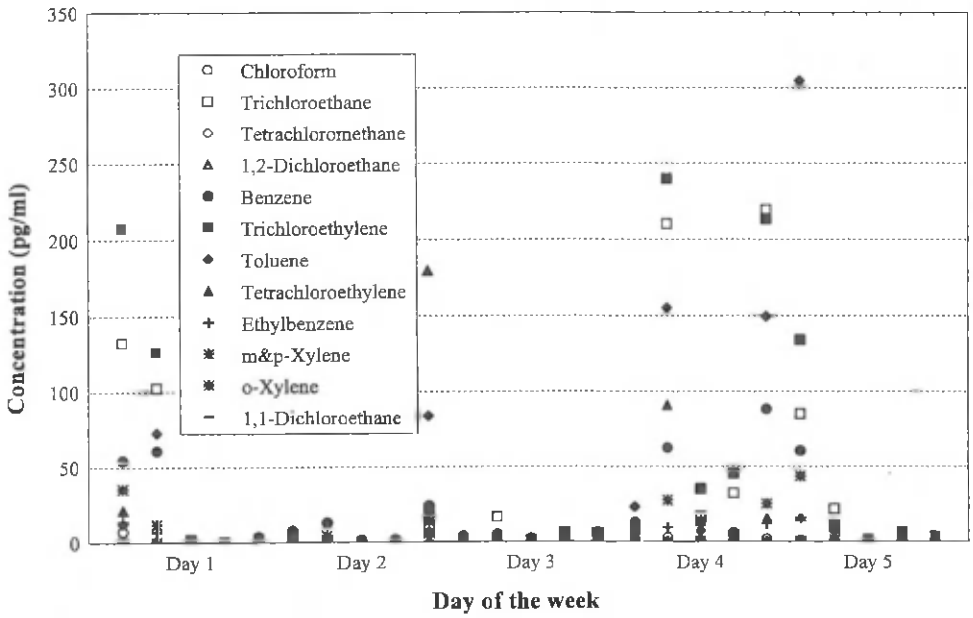

Fig. 2. Variability of the background concentration in laboratory air over a period of one week.

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 is 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.

3.2. Sample treatment and foaming

Sample treatment involves dissection of the organism to obtain the edible tissue and processing of the tissue. In the literature, sample treatment 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 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 R.S.D.s for the former procedure (Table 2). Moreover, trichloroethylene could apparently only be purged out of the tissue after homogenisation and there was a much higher yield for chloroform.

Initially, severe sample foaming was observed which 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, nonvolatile 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 the flow-rates (40 ml/min) and with the equipment they used. However, using similar conditions, a glass wool barrier could not contain 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% (w/w) 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 level 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

Table 2
Effect of homogenisation on the analysis of fish tissue samples

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

n=Number of analyses, R.S.D.=relative standard deviation.

mechanical barrier this eventually turned out be the best way to prevent foam from reaching the trap.

Unfortunately, reducing the purge flow has 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 h with the actual values depending on the volume of the purging device [11–13]. 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 min with an optimum around 40 min for the MAHs and around 30 min for the CHCs (Fig. 3). For the simultaneous determination of both groups of compounds a purge time of 30 min was selected.

3.3. Comparison between off-line and on-line determination

All analyses were initially performed off-line. To study the feasibility of on-line PT, the vessel used for the off-line determination was coupled to the sparger of the Tekmar (Fig. 4). Since this is the only change in the setup, the experimental conditions could be kept the same. The main focus was therefore on the background obtained with the on-line system. To this purpose, a blank water sample was analysed several times using both setups. The experimental results are shown in Table 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 R.S.D. value for the on-line method. For all other analytes, the precision was the same or much better with the

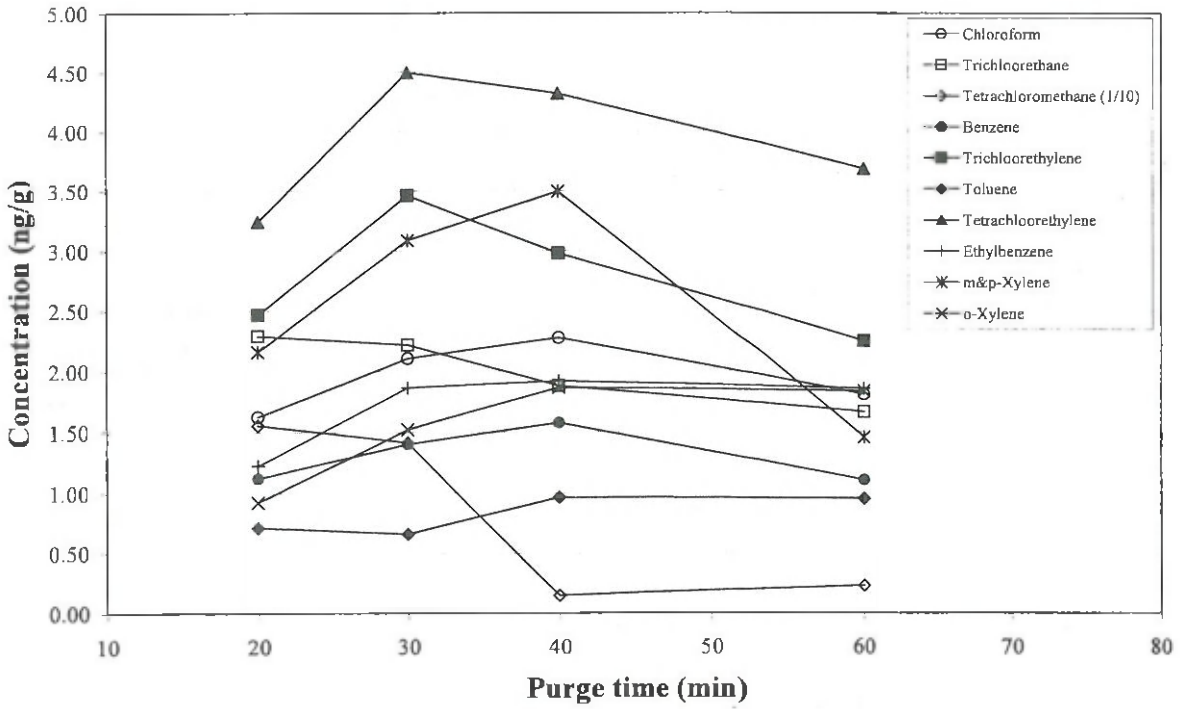

Fig. 3. Dependence of recovery on purge time.

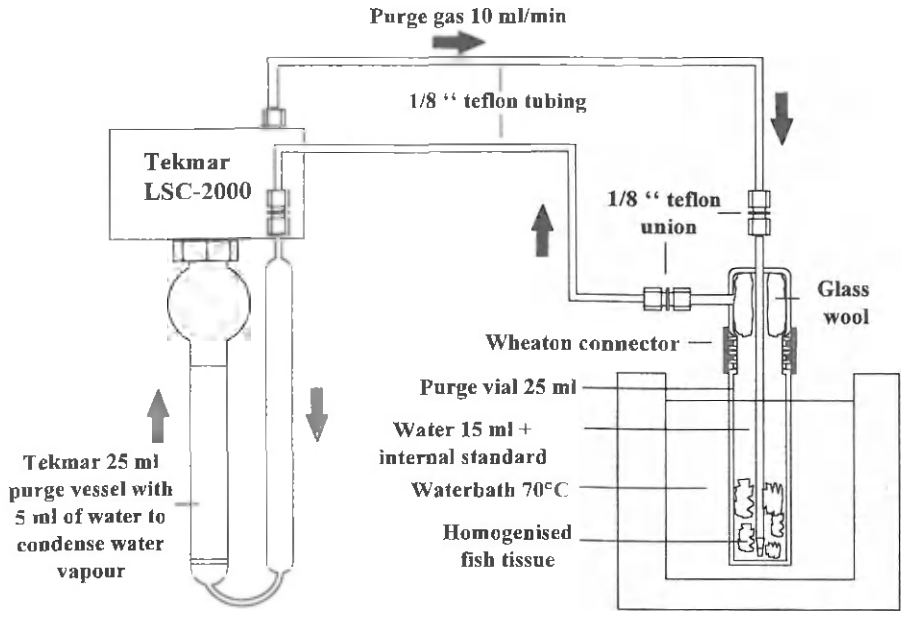

Fig. 4. Schematic of the on-line setup.

on-line setup. As a result, it was selected for all further work.

3.4. Analytical data

The LOD of the VOCs was calculated using two methods. Considering the variability of the daily blank values, the LOD was defined as the amount corresponding to the blank plus three S.D.s of the blank. Since, in practice, the R.S.D.s of the blank are around 30%, the LOD was set at two times the blank value. A similar approach was previously reported in the literature [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 4. They are seen to range from 5 to pg/g 200. This work aimed at a detection limit of 100 pg/g or better. With one exception this goal has

Table 3
Average peak height, relative standard deviation (R.S.D.) and ratio between the averages of analyses blank water with the off-line and on-line setup

Compound	Off-line ($n=6$)		On-line ($n=5$)		Ratio
	Average	R.S.D. (%)	Average	R.S.D. (%)	
Chloroform	210 000	37	1900	36	114
Trichloroethane	2900	14	0	–	≫
Tetrachloromethane	nd	–	nd	–	–
Benzene	265 000	41	349 000	16	0.7
Trichloroethylene	1600	26	1044	127	1.5
Toluene	183 000	37	20 600	12	9
Tetrachloroethylene	48 700	36	nd	–	≫
Ethylbenzene	196 000	30	5000	12	39
m- and *p*-Xylene	284 000	26	11 000	17	26
o-Xylene	66 000	25	1400	20	47

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

Table 4
LODs, within-day precision and recovery for the analysis of VOCs in 10-g marine biota samples

Compound	LOD (pg/g)	R.S.D. (%) (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- and *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

been reached, 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 4. The R.S.D. values varied between 8% and 25% for all VOCs except chloroform (36%). R.S.D.s reported in the literature for the various methodologies vary between 2 and 30%, and, specifically for PT techniques, R.S.D.s are between 5 and 20%. The rather close similarity, between the various sets of R.S.D.s strongly suggests that they are the best available for PT 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.

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 h at room temperature and in the dark prior to analysis. The experimental results of the analysis are included in Table 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 concentration levels 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.

3.5. 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 Fig. 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

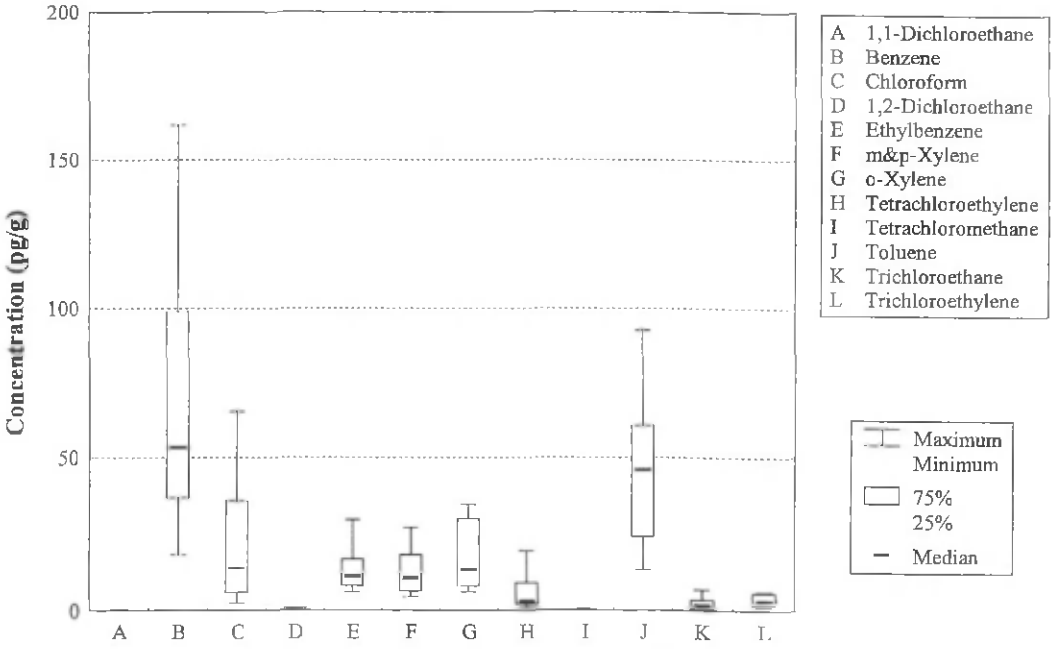

Fig. 5. Background concentrations of analytes of interest over a period of 22 weeks.

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 PT 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 laboratory-made library (Fig. 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 (Fig. 6). There were two types of exception, distorted mass spectra as a result of water breakthrough (99%) and coeluting 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 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). Rerunning 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%.

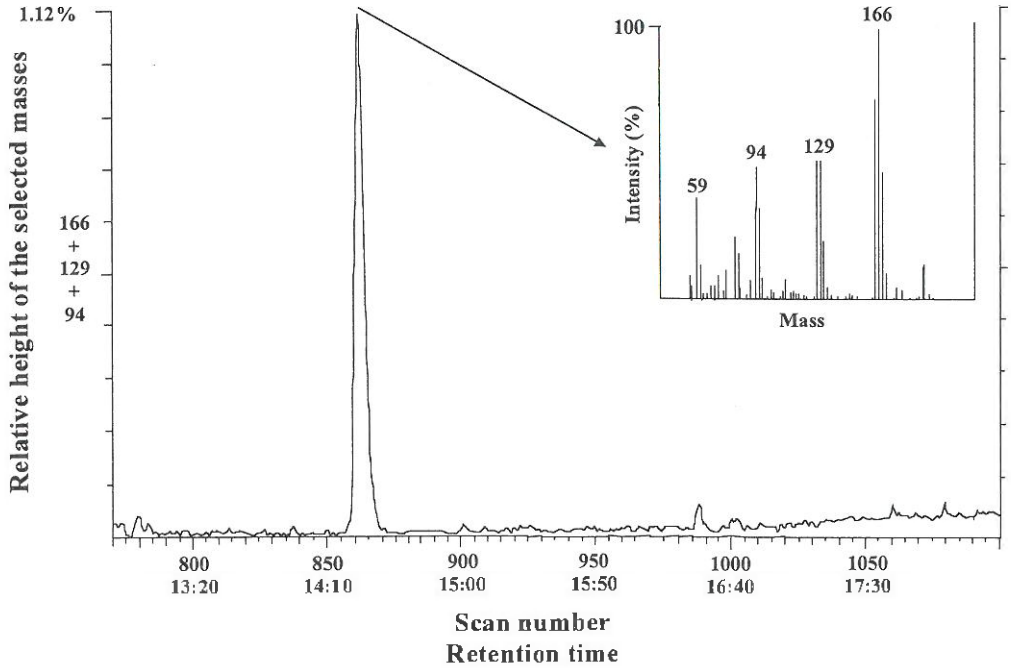

Fig. 6. Selected ion chromatogram and mass spectrum for tetrachloroethylene (80 pg/g) in muscle tissue of whiting.

3.6. Analysis of marine samples

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 concentration levels of VOCs actually present and the range of concentrations within a population. The results are illustrated in Fig. 7. Although, the range of concentrations within a tissue was considerable, with R.S.D.s 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 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 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 (Fig. 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 mass (wwt) were found for muscle tissue and around 1–6 ng/g wwt for liver tissue. 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

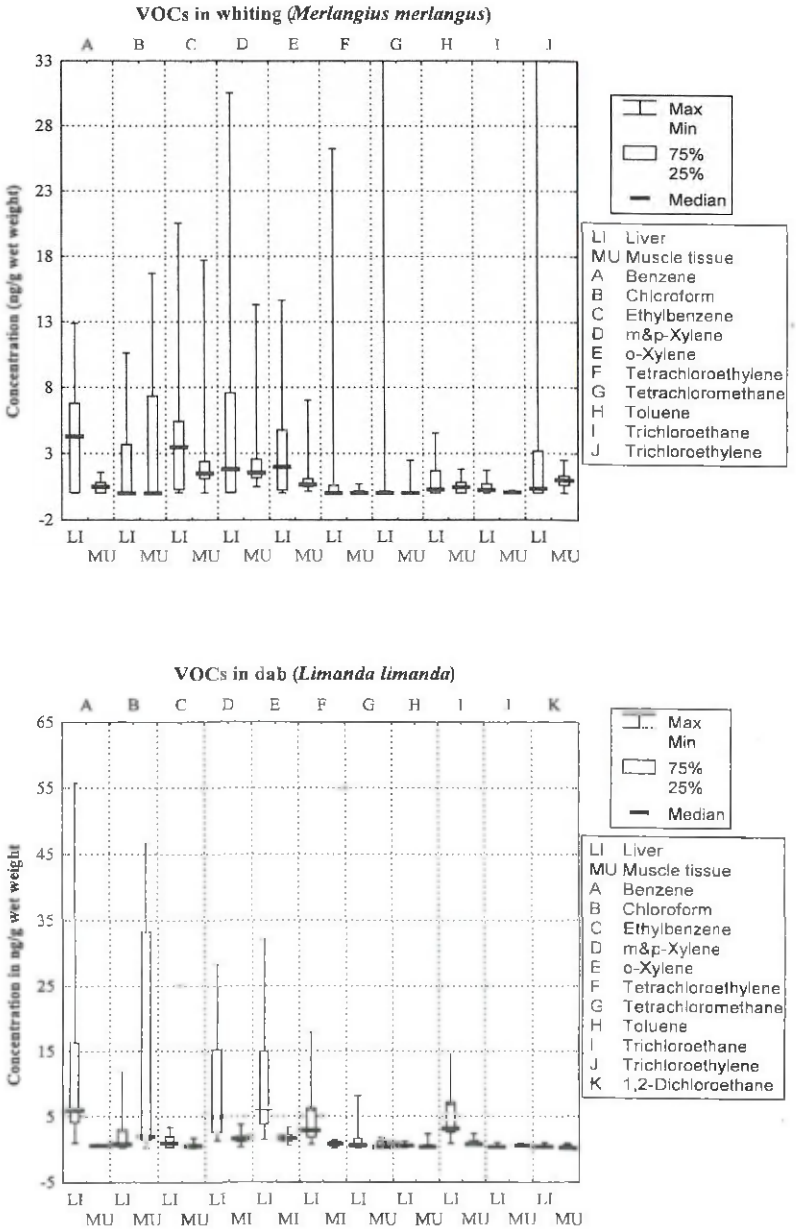

Fig. 7. Concentrations of VOCs in muscle tissue and liver of whiting (top) and dab (bottom).

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. Similar high values have

Table 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)	R.S.D. (%)	KS distance	KS *P*-value
Whiting muscle tissue						
Benzene	13	4.4	4.0	46	0.1508	>0.10*
Chloroform	10	41.7	198	83	0.4167	0.062*
m- and *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	0.3855	0.0039
Tetrachloroethylene	12	0.2	0.3	64	0.2444	>0.10*
Tetrachloromethane	4	0.7	3.2	191	0.4178	>0.10*
Toluene	15	2.6	3.4	63	0.1875	>0.10*
Trichloroethane	9	1.0	1.2	79	0.268	>0.10*
Trichloroethylene	20	4.6	4.6	58	0.1266	>0.10*
Whiting liver tissue						
Benzene	18	4.86	5.96	68	0.146	>0.10*
Chloroform	11	3.66	4.66	78	0.155	>0.10*
m- and *p*-Xylene	23	3.46	4.90	126	0.247	>0.10*
Ethylbenzene	19	3.37	6.67	134	0.232	>0.10*
o-Xylene	21	2.20	3.60	114	0.220	>0.10*
Tetrachloroethylene	9	0.89	4.25	203	0.420	0.0839*
Tetrachloromethane	10	76	154	128	0.283	>0.10*
Toluene	12	1.63	2.10	68	0.195	>0.10*
Trichloroethane	20	0.22	0.51	103	0.256	>0.10*
Trichloroethylene	16	1.16	29.3	195	0.411	0.0091
Dab muscle tissue						
Benzene	16	0.52	0.54	20	0.1578	>0.10*
Chloroform	17	1.90	14.3	126	0.3292	0.0502*
m- and *p*-Xylene	16	0.40	0.52	86	0.1874	>0.10*
Ethylbenzene	18	1.66	1.71	47	0.1449	>0.10*
o-Xylene	16	1.66	1.75	43	0.1805	>0.10*
Tetrachloroethylene	16	0.79	0.77	42	0.1108	>0.10*
Tetrachloromethane	13	0.30	0.62	92	0.3148	>0.10*
Toluene	12	0.30	0.54	113	0.2609	>0.10*
Trichloroethane	14	0.73	0.97	53	0.2601	>0.10*
Trichloroethylene	6	0.48	0.53	39	0.3129	>0.10*
Dab liver tissue						
Benzene	19	5.64	11.8	116	0.2567	>0.10*
Chloroform	20	0.76	2.45	136	0.2722	>0.10*
m- and *p*-Xylene	16	0.99	1.26	78	0.1572	>0.10*
Ethylbenzene	20	4.87	9.36	95	0.25	>0.10*
o-Xylene	20	6.19	9.98	90	0.2012	>0.10*
Tetrachloroethylene	20	2.81	4.78	96	0.2725	>0.10*
Tetrachloromethane	20	0.66	1.27	137	0.2725	>0.10*
Toluene	8	0.51	0.57	55	0.2148	>0.10*
Trichloroethane	20	3.04	4.89	77	0.224	>0.10*
Trichloroethylene	17	0.25	0.36	69	0.2687	>0.10*

n=Number of values used for the calculation (i.e., number of fish), R.S.D.=relative standard deviation, *=significant, KS=Kolmogorov–Smirnov.

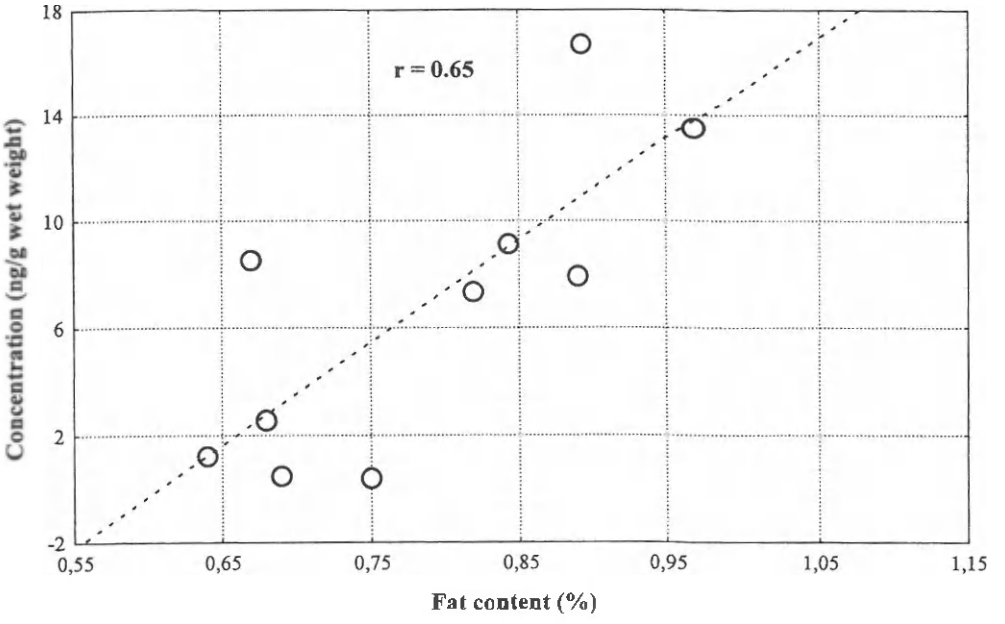

Fig. 8. Relationship between chloroform concentration and fat content in muscle tissue for whiting. See Section 3.6 for details.

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*) and 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). Finally, Ferrario et al. [12] reported benzene concentrations up to 1030 ng/g in killifish (*Fundulus* sp.). Finally, the concentration levels 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 VOCs exhibit little or no tendency to bioconcentrate [17]. The similarity between the concentration levels 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.

4. 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 Van Langenhove [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 No. 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 concentration levels 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.

Acknowledgements

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