Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium)

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

Aim of the investigation

Volatile organic compounds (VOCs) are well-known atmospheric contaminants that are frequently determined in air, drinking water, fresh water, effluents and soils (Anderson et al.,1 Sweet and Vermette,2 Dewulf et al.). Most representatives of the group are important industrial compounds with a high annual production (OECD) which can be anywhere in the range from several hundred thousand tonnes for e.g. tetrachloromethane, to more than 10 billion tonnes for benzene (Howard,3 WHO4). In Belgium, the emissions of the chlorinated hydrocarbons (CHCs) chloroform, 1,1,1-trichloroethane, and tri- and tetrachloroethene, exceed those of e.g. lead, lindane and atrazine (Anon 7). Moreover, benzene, toluene, ethylbenzene and the xylenes (BTEX) are important additives to unleaded gasoline and are present in crude oil. Several international organizations therefore regard VOCs as compounds with a high research priority (Ministerial Declaration of the 3rd International Conference on the Protection of the North Sea, Ministerial Declaration of the 4th International Conference on the Protection of the North Sea5).

The low values of the logarithm of the octanol–water partition coefficients (log Kow) of the VOCs, typically 1–2, led to the general belief that bioconcentration should be considered insignificant (Howard3,5 WHO6). As a result, the presence of VOCs in organisms was studied by a limited number of research groups only and there are few recent findings in the literature (Roose and Brinkman8). The considerable analytical problems associated with the determination of these compounds in environmental matrices, specifically in biota, can be regarded as another reason for the lack of information. It was somewhat surprising, therefore, that recent studies showed the general presence of a number of important VOCs in the tissue of marine organisms from different levels of the food chain (Roose and Brinkman8). It was also found that the concentration levels in marine organisms were up to a thousand times higher than those in the surrounding water. The bioconcentration factors calculated from these data were generally higher than those reported in the literature. A possible explanation is the continuous exposure of organisms to low or even undetectable levels of these compounds in the water column. Determination in the water column alone is, therefore, insufficient.

Aquatic organisms can be, and have been, used successfully to monitor contaminants in various ecosystems, especially when the concentrations of these compounds in the water column are extremely low (de Boer and Hagel9,10). For an organism to become a potential biomonitor or sentinel organism, several criteria should be fulfilled. First and foremost, the organism should reflect the actual condition of the surrounding water column. This implies that it should show little or no migratory behaviour and that the species should commonly occur in the area under investigation. The yellow eel, Anguilla anguilla L., appears to be a most adequate indicator organism for the pollution status of freshwater environments. Eels are benthic fish which have a widespread geographical distribution. They are carnivorous organisms that predate mainly on insect larvae, worms, crustaceae, snails, mussels and fish, in particular small bottom-dwelling species. Moreover, the yellow eel has a high proportion of lipids in its body, which facilitates the accumulation of lipophilic contaminants. The accumulation is further promoted by the fact that no spawning occurs during the eels’ stay in inland waters. The eel is also essentially sedentary and normally does not migrate (de Boer and Hagel12). The same authors showed that the yellow eel reflects rapid changes in the concentrations of organic contaminants in the surrounding water.

In this study, a limited number of eels, which have been sampled as part of a routine monitoring programme, were analysed by means of a previously developed method (Roose...
and Brinkman\textsuperscript{13} for their VOC content. The study is intended as a screening exercise to get an impression of the concentration levels of VOCs in the yellow eel, the potential environmental hazard and the possibility of the future use of the yellow eel as an indicator organism.

Materials and experimental procedures

Samples and sampling

Eels were sampled by means of either electrofishing along river banks, fyke fishing or seine netting. Samples were initially collected in the framework of the fish stock assessment programme of the Institute for Forestry and Game Management which aims at monitoring fish and the biotic integrity of riverine and lacustrine waters all over Flanders. The samples were subsequently analysed for their PCB, organochlorine pesticide and heavy metal content, and in the framework of this study, for the presence of VOCs. An overview of the sixteen inland water stations is given in Table 1. The stations can be characterized as rivers (width \textgreater{} 10 m width, 4 stations), brooks (width \textless{} 10 m, 2 stations), canals (8 stations) and enclosed water bodies such as ponds (6 stations). They are located in rural as well as in densely populated industrial areas (Fig. 1). Twenty eels were selected from the 30–70 cm size range (Table 1).

Lipids were measured by total lipid extraction following Bligh and Dyer.\textsuperscript{14}

Analytical methodology

A detailed description of the analytical methodology is given elsewhere (Roose and Brinkman\textsuperscript{13,15}). Briefly, biological tissue is first homogenised at 0 °C in an ultraturrax blender and transferred to a 40-ml vial. After addition of 25 ml of water and

<table>
<thead>
<tr>
<th>No.</th>
<th>Location</th>
<th>Type of water</th>
<th>Surroundings</th>
<th>River basin</th>
<th>Length/cm</th>
<th>Weight/g</th>
<th>Lipid content (%)</th>
</tr>
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<td>Leie</td>
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<td>Rural</td>
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<td>Demer</td>
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<td>A, Poppel</td>
<td>Brook</td>
<td>Rural</td>
<td>Maas</td>
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<td>Pond</td>
<td>Industrial</td>
<td>Benedenschelde</td>
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<td>181</td>
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<td>Industrial</td>
<td>Nete</td>
<td>50</td>
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<td>Brook</td>
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<td>Maas</td>
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<td>16</td>
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<td>Darse, Vilvoorde</td>
<td>Canal</td>
<td>Industrial</td>
<td>Dijle-Zenne</td>
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<td>191</td>
<td>31</td>
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<td>Kanaal Beverlo, Leopoldsburg</td>
<td>Canal</td>
<td>Industrial</td>
<td>Nete</td>
<td>59</td>
<td>321</td>
<td>21</td>
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</tbody>
</table>

*Also see Fig. 1.

Fig. 1 Sampling locations in the region of Flanders (Belgium) (Source: OC Gis Vlaanderen and AMINAL, Water Section; see also Table 1).
the internal standard (1,1,1-trifluorotoluene), the homogenate is treated for 20 min at 0 °C in an ultrasonic bath to further disrupt the tissue. The glass vessel is then connected to a Tekmar (Cincinnati, OH, USA) LSC 2000 purge-and-trap apparatus coupled to a Finnigan Magnum (Finnigan, San José, CA, USA) gas chromatograph-mass spectrometer (GC-MS). The volatiles are forced out of the tissue by purging with a stream of helium while heating at 70 °C, and trapped onto a Vocarb 4000 sorbent trap (Supelco, Bellefonte, PA, USA). After purging, the trap is backflushed while being rapidly heated to 250 °C, the analytes are desorbed and, next, trapped in a cryofocusing module (−120 °C) connected to the GC column (J&W, Folsom, CA, USA, DB-VRX, 60 m, 0.25 mm id, 1.4 μm film). The analytes were injected into the column by rapidly heating the module from −120 °C to 200 °C in 0.75 min. Temperature programming of the GC and data acquisition were started simultaneously. The temperature of the GC oven was held at 35 °C for 6 min and then linearly increased to 200 °C at 4 °C min⁻¹. This temperature was then held for 4 min. Helium with an inlet pressure of 16 psi was used as the carrier gas. The ion-trap detector was operated in the electron ionisation (EI) mode with the multiplier voltage set at 2400 V, the axial modulation (A/M) amplitude at 3.5 V and the emission current at 12 mA. 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 m mas/100 amu and a background mass of 55 amu were selected.

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

**Results and discussion**

**VOC concentrations in eel**

The twenty eels from the various inland water bodies were analysed for a total of 52 VOCs which are listed in Table 2.

Table 2 Set of 52 VOCs studied and relevant analytical information

<table>
<thead>
<tr>
<th>Sequence number</th>
<th>Compound</th>
<th>Masses (m/z)</th>
<th>Retention time/min</th>
<th>LOD ng g⁻¹</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>trans-1,2-Dichloroethene</td>
<td>61/96/98</td>
<td>2:24</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>1,1-Dichloroethene</td>
<td>63/83/97</td>
<td>3:26</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>cis-1,2-Dichloroethene</td>
<td>61/96/98</td>
<td>6:04</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>2,2-Dichloropropane</td>
<td>77/79/97</td>
<td>7:14</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>Bromochloromethane</td>
<td>130/128/49</td>
<td>6:56</td>
<td>0.1</td>
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<tr>
<td>6</td>
<td>Chloroform</td>
<td>83/85</td>
<td>7:17</td>
<td>0.3</td>
</tr>
<tr>
<td>7</td>
<td>1,1,1-Trichloroethane</td>
<td>97/61/99</td>
<td>11:40</td>
<td>0.05</td>
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<tr>
<td>8</td>
<td>Tetrachloromethane</td>
<td>117/119</td>
<td>14:24</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>Dichloropropane</td>
<td>39/110/77</td>
<td>13:20</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>Benzene</td>
<td>78</td>
<td>15:04</td>
<td>0.2</td>
</tr>
<tr>
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<td>1,2-Dichloroethane</td>
<td>62/64</td>
<td>11:12</td>
<td>0.01</td>
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<tr>
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<td>Trichloroethene</td>
<td>130/95/60</td>
<td>20:34</td>
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<td>Di bromomethane</td>
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<td>18:53</td>
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<tr>
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<td>Bromochloromethane</td>
<td>83/85/47</td>
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<tr>
<td>I.S.</td>
<td>Trifluorotoluene</td>
<td>146/127/96</td>
<td>23:00</td>
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<td>16</td>
<td>cis-1,3-Dichloropropane</td>
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<td>25:14</td>
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<td>91</td>
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<td>75/110/39</td>
<td>27:49</td>
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</tr>
<tr>
<td>19</td>
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<tr>
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<tr>
<td>28</td>
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<td>91/105/106</td>
<td>37:30</td>
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<td>o-Xylene</td>
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<td>Styrene</td>
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<td>Isopropylbenzene</td>
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</table>

*In order of relative abundance. °For a 40-g sample with extracted ions. †Internal standard. ‡Not determined.
Compounds were identified on the basis of their mass spectrum and their concentrations were calculated by using at least two selected ion masses (exceptions: benzene and toluene). As an illustration, a full scan GC-MS chromatogram and a selected ion chromatogram for tetrachloroethene in eel sample No. 5 are shown in Fig. 2. Detection limits (LODs) in the selected-ion mode for 40 g samples were calculated on the basis of a signal-to-noise ratio of 3 or 3 times the standard deviation of the blank. They varied between 0.01 ng/g wet weight (1,2-dichoroethane, 1,1-dichloroethane and tetrachloromethane) and 6 ng/g wet weight (trichlorobenzene) depending on the background levels and the amount of sample.

All relevant data are presented in Table 3. The results show that about half of the target VOCs, i.e. 25 out of 52, were detected in one or more eel samples. A detailed breakdown of the results is presented in Fig. 3 which shows the percentage of samples that was positive for a given VOC. One striking observation is that the BTEX compounds were present in all

Table 3

Concentrations (ng/g ww) of VOCs detected in freshwater eels

<table>
<thead>
<tr>
<th>VOC</th>
<th>Station</th>
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<td>1.6</td>
<td>5.4</td>
<td>6.9</td>
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<tr>
<td>1,2,3-Trichlorobenzene</td>
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<td>1.7</td>
<td>5.4</td>
<td>10</td>
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</tbody>
</table>

*Values below LOD, as given in Table 2, are reported as "—". aFor locations, see Table 1.*
samples. A further five compounds, chlorobenzene, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, naphthalene and chloroform, were present in 70–90% of all samples, and a 35–60% positive score was obtained for nine VOCs, 1,3,5-trimethylbenzene, isopropylbenzene, tetrachloroethene, 1,2,4-trimethylbenzene, 1,2-dichlorobenzene, hexachlorobutadiene, 1,2-dichloroethane, \( p \)-isopropyltoluene and 1,2,3 trichlorobenzene. The other VOCs were found in 20% of the samples or less.

The concentrations of the VOCs that were detected varied considerably, as is graphically illustrated by the box and whisker plot of Fig. 4. The median concentrations typically were 1–10 ng/g, ranging from 0.5 ng/g for isopropylbenzene to 14 ng/g wet weight for tetrachloroethene. High concentrations of over 30 ng/g were found for twelve of the VOCs, with a staggering 700 ng/g wet weight for 1,2-dibromo-3-chloropropane in eel from the Albertkanaal, Langerlo, as the maximum. Extensive statistical testing, such as principal component analysis, seemed inappropriate because of the limited number of statistical cases. Nonetheless, a correlation analysis was performed for the concentrations of the reported VOCs. While

Fig. 3 Percentage of positive samples for the detected VOCs in order of abundance.

Fig. 4 Box and whisker plot of the detected VOCs for all eel samples, with from left to right: (6) chloroform; (7) 1,1,1-trichloroethane; (10) benzene; (11) 1,2-dichloroethane; (17) toluene; (20) tetrachloroethene; (24) chlorobenzene; (26) ethylbenzene; (27) \( m \)-xylene; (28) \( p \)-xylene; (32) isopropylbenzene; (36) \( n \)-propylbenzene; (38) 1,3,5-trimethylbenzene; (41) 1,2,4-trimethylbenzene; (43) 1,3-dichlorobenzene; (44) \( p \)-isopropyltoluene; (45) 1,4-dichlorobenzene; (47) 1,2-dichlorobenzene; (48) 1,2-dibromo-3-chloropropane; (49) 1,2,4-trichlorobenzene; (50) hexachlorobutadiene; (51) naphthalene; (52) 1,2,3-trichlorobenzene.
no significant correlation was found for any of the other VOCs, the BTEX compounds were found to correlate extremely well with each other, with correlation coefficients of between 0.77 and 0.98, and on an average, 0.89 (p < 0.005, n = 20).

The fairly high concentrations found in this study do not come as a complete surprise: the general picture agrees with earlier observations which, actually, triggered this work. The earlier studies showed that various VOCs were present in both marine organisms and in eel from the Scheldt estuary (Roose and Brinkman11,13). In Fig. 5 the concentration levels of a number of priority VOCs in marine organisms from the Belgian coastal water are compared with the results of this study. The concentrations of the chlorinated hydrocarbons (CHCs) are seen to be generally significantly lower in marine fish than in eel from inland waters. This is the case even for lipid-rich tissues such as the liver. Literature data on CHCs in eels are very limited. An exception is the overview by Howard10 which reports tetrachloroethene concentrations in American eels of 105–250 ng g$^{-1}$ that are at least an order of magnitude higher than in marine organisms. This is similar to what is observed here. Especially for this analyte, the observed median concentrations are a lot higher in eels than in marine fish. Tetrachloroethene has a limited bioconcentration capacity and accumulation occurs in the lipid-rich tissues of both man and animals (WHO16). The higher observed levels in eels are therefore more than likely the result of a higher exposure of freshwater organisms to this compound. The same also seems to apply to the other CHCs, although to a lesser extent. The difference is probably related to differences in uptake and metabolism rates and the lower bioconcentration capacity of the other CHCs.

In contrast to the CHCs, median concentration levels of BTEX in eels are more or less the same as those found in the liver of marine fish, with the exception of, perhaps, toluene. In contrast to CHCs, BTEX emissions are not solely related to industrial processes, i.e. local sources. BTEX were indeed found at all sampling locations and the variability of the data is somewhat less than for the other VOCs (Fig. 4). BTEX are common constituents of diesel oil and many petrochemical products, and are emitted in the exhaust gases of combustion engines (Howard,5,10 Crookes et al.17). This fits well with the observed correlation between the BTEX compounds and is in

![Fig. 5 Comparison of the concentrations of selected (a) CHCs and (b) BTEX in tissues of marine species and freshwater eels.](image)
line with our earlier observations on VOCs in marine organisms (Roose and Brinkman\(^1\)). In that study, the observed correlation for these compounds was related to this common source and it was suggested that the principal source of BTEX in marine organisms is the use of fossil fuel. Dewulf \(^3\) observed higher levels of MAHs (monoaromatic hydrocarbons) than of CHCs in water and air samples from the same region and attributed this also to anthropogenic emissions from marine traffic in this coastal area. The same group also carried out an extended study of VOCs in the water column of the estuary of the Scheldt river and found similar results for BTEX in the water column (Dewulf \(^3\)). These authors observed significant correlations between the various BTEX and a more uniform distribution of the concentrations throughout the estuary compared to CHCs. BTEX concentrations in this study were also of the same order of magnitude as in the marine environment, which was not the case for CHCs. These observations support the hypothesis that contamination by BTEX is of a rather diffuse nature which, in its turn, supports the conclusion that the use of fossil fuel in, e.g. traffic, is the major source of BTEX.

Spatial distribution of VOCs and eel as a biomonitor

The current database is too limited to allow an analysis of the spatial distribution for all VOCs included in this study. Such a comparison is justified only for the most prominent VOCs. That is, the comparison was limited to chloroform and tetrachloroethene, and the BTEX compounds. The latter are considered as a group based on the correlation discussed above, and are represented by their sum. Fig. 6 gives an overview for the selected VOCs per sampling station and river basin. The patterns for eels collected at the same locations (Albertkanaal, Grensmaas, Kanaal Leuven-Dijle) are closely similar both with regard to the concentrations and their ratios. As regards the different river basins, the VOC concentrations in eels from highly industrialized and populated regions (Dender, Dijle-Zenne and Nete basins) are higher. This is especially true for BTEX. The high concentrations observed at the Groot-Zuunbekken station can possibly be explained by the fact that this is a pond in a densely populated and industrialized area, which is in the vicinity of a tributary of the Zenne river, the Zuunbeek, which is biologically dead. Probably, water from the brook entering the pond explains the observed results. Since there is little exchange with surrounding water masses, VOCs are lost probably only as a result of evaporation. As this is a dynamic process, it would indicate a constant high level of input into that water body. In marked contrast, eels from rural locations, such as the A at Poppel, have a significantly lower body burden.

Recent data for the concentrations of the same VOCs as were studied here in the water columns of Flemish rivers show that these are generally below the LODs of the analytical techniques used, i.e. 0.05–2 \(\mu\)g l\(^{-1}\). That is, they are below the current water-quality criteria of the Flemish government, which are set at a median value of 2 \(\mu\)g l\(^{-1}\) for total VOCs and 1 \(\mu\)g l\(^{-1}\) for each individual VOC (VMM, \(^{18}\) VMM, unpublished results). Not surprisingly, the VOCs that were detected in the water columns are the same as the most prominent ones in this study and the highest concentrations are also found in the Dijle-Zenne basin. Taking into account that the bioconcentration factor (BCF), viz. the ratio of the concentrations of an analyte in the organism and the water, is between 1 and 90 for most VOCs (Isnard and Lambert, \(^{19}\) Freitag \(^{20}\) et al., Howard\(^5\)), the concentration levels found in eels are not surprising. For instance, if the LODs of the BTEX compounds in water are taken as the actual concentrations (0.2–0.4 \(\mu\)g g\(^{-1}\)), concentrations of 20–40 ng g\(^{-1}\) would be expected in eels if an estimated log BCF of 2 is used (Howard\(^5\)). As can be observed from Fig. 4, median values of approx. 10 ng g\(^{-1}\) were found for the various sampling stations in our study. This allows the conclusion that concentrations in eels indeed reflect the concentrations in the water column. Moreover, the—admittedly, limited—information presented above shows that eel
samples from the same location have similar patterns and VOC concentration levels. There is evidence to suggest that once contaminants are stored in the lipid, they will not be metabolised and become resident. Also because eels do not spawn during their stay in inland waters, the observed concentrations are valuable for time-trend analysis, and, because the eel is essentially sedentary and normally does not migrate, concentration data should allow the comparison of different river systems. An additional advantage is that yellow eels are known to reflect rapid changes in the concentrations of organic contaminants in the surrounding water (de Boer and Hagel12). In summary, the yellow eel Anguilla anguilla L. can be considered as a potential biomonitor or sentinel organism for VOCs.

Hazard assessment

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

Table 4 shows the HC5 values for some selected VOCs and their concentrations measured at the various sampling stations. The results show that in no case is the HC5 exceeded. Moreover, the experimentally determined concentrations are several orders of magnitude lower than the HC5. One may therefore assume that, in all likelihood, this is also true for those VOCs for which no HC5 data are available. On the other hand, one should note that the hazard assessment does not take into account synergistic and, thus, more damaging effects. To quote an example, the eel from Groot Zuunbekken, with the highest concentrations of VOCs, did have an abnormally low lipid content, viz. 9% compared to an average of 25%. Nevertheless, more definite statements regarding long-term effects cannot, as yet, be made because the dataset is far too small and the calculation of the HC5 is only one approach amongst several and needs to be further evaluated. That is, additional research, especially with regard to the long-term consequences of small doses of VOCs is urgently required and the use of eels as sentinel organisms for VOCs should be studied in more detail.

Conclusions

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

The present exercise indicates that the VOC concentrations in eels reflect the actual concentrations in their environment. Also, if the BCFs and the concentrations in the water column

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**Table 4** Comparison between observed VOC concentrations (ng g\(^{-1}\)) and HC5 values (ng g\(^{-1}\)) calculated according to Van Leeuwen et al.21

<table>
<thead>
<tr>
<th>Location</th>
<th>Benzene</th>
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<th>p-Xylene</th>
<th>o-Xylene</th>
<th>Chloroform</th>
<th>Tetrachloroethene</th>
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\(^*{———}^*\) Values below LOD (see Table 2).
are taken into account, the observed levels are well in line with expectations. In other words, the eel is a potential biomonitor or sentinel organism for VOCs and further study is justified. This should include extended sampling at given locations and a more in-depth study of the behaviour of VOCs in the organism. For the rest, a follow-up study should be sufficiently wide-ranging to allow evaluation of the long-term consequences of small doses of VOCs and their synergistic effects.

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References