INTRODUCTION

Risk assessment of mixtures

Risk assessment is usually based on a single chemical approach. Polluted sites, effluents, and streams, however, usually contain complex mixtures of sometimes hundreds of different compounds. A single chemical approach is obviously not sufficient for such sites. For example, Hendriks et al. [1] showed that the concentrations of individual chemicals could explain only 10% of the total toxicity of concentrated surface water samples. In addition to a compound-specific analytical chemical approach, other group parameters or assays are often used in the evaluation of complex mixtures, for example: chemical group parameters (e.g., adsorbable organic halogen [AOX] content and adsorbable organic phosphorus content); biochemical group parameters (e.g., induction of ethoxyresoru®n-O-deethylase); and bioassays (e.g., Daphnia magna, Microtox®, and fish tests). In many cases, extraction and concentrations steps (e.g., extraction using XAD) precede these assays. Toxicity identification evaluation (TIE) [2,3] is an example of a more systematic approach, the objective of which is to identify those chemicals that are of major toxicologic concern in a particular polluted site or stream.

A more complete overview of present mixture toxicity parameters is given in a recent report from van Loon and Hermens [4]. All these group parameters or approaches have their advantages and drawbacks. The group parameter AOX, for example, will supply information on the total content of halogenated organics, but the outcome of an AOX determination often is not directly related to toxic effects. For example: pentachlorobenzene is 250 times more toxic than is monochlorobenzene, although the chlorine content differs only by a factor of five. Analytical chemical data will give information about the presence of major pollutants, but only a small fraction of the chemicals in surface waters can be identified and quantified [1]. Moreover, effect data for the identi®ed compounds are often not available [1]. A TIE analysis is able to identify major toxic components in a polluted site. However, effects may also be caused by the presence of a mixture of many different chemicals at relatively low concentrations. Joint effects of such mixtures, in particular of mixtures of chemicals with a similar mode of action, are often concentration additive. Research into quantitative structure±activity relationships has resulted in the distinction of several modes of action classes, the most extensive of which is the class of chemicals that possess a nonspeci®c mode of action (narcosis) [5–10]. It is estimated that more than 50% of the major contaminants are only nonspeci®cally acting chemicals; well-known examples are halogenated and nonhalogenated aromatic and aliphatic hydrocarbons (e.g., xylenes, benzenes, toluenes, alkanes) [5–10]. Because of the additive effect of chemicals with a similar mode of action (a toxicologic class), there is a need for group parameters that supply information on total concentrations or total (combined) effects within such a toxicologic class. In this paper, we have applied such a parameter for the class of chemicals that act by narcosis.

A group parameter for bioaccumulation and baseline toxicity

The effects of narcosis-type chemicals are well known to be completely concentration additive [11–18]. Intrinsically, these chemicals are all equally toxic. In other words, body burdens elicitating at a certain effect are the same for all compounds within this toxicologic class [8,19–22], or using a well-known term from research on dioxin and polychlorinated biphenyls, the toxic equivalence factor is 1.0 for all these chemicals. The differences in aqueous effect concentrations of chemicals within this class are only due to differences in bioconcentration factors [19–21].
The concept of critical body residues was introduced by McCarty [19] and has been applied in several other studies [19–24]. Critical body residues for baseline toxicity at a few well-known endpoints or effects are given in Table 1. The no-effect body residues are given here on a molar and lipid weight basis. In a risk assessment, experimental data for body residues of individual compounds (BR) or for mixtures of chemicals, here defined as total body residues (TBRs), can be compared with these no-effect body residues.

It is easy to define the parameter TBR, but its determination is more complicated. Clearly, one strategy is to identify and quantify all individual chemicals in a complex mixture and to add these concentrations, but this is an almost impossible task. In our laboratory a simple method for estimating measuring TBRs has been developed, not by focusing on individual chemicals but by quantifying total molar concentrations of mixtures in one measurement. Results of this basic research have been published by Verhaar et al. [25] and Van Loon et al. [26].

Estimation of TBRs

The experimental procedure for estimating TBRs is based on two important features: a biomimetic extraction procedure and the determination of total molar concentrations.

The biomimetic extraction procedure. Measurement of TBRs can in principle be carried out in biota, but a very extensive clean-up would be needed to purify the samples from compounds such as proteins and lipids and such measurement is therefore not practical. Instead of working with biota, we have chosen to use a solid-phase extraction on a hydrophobic phase in order to mimic the uptake by organisms. This biomimetic extraction has been extensively described by Verhaar et al. [25]. A biomimetic extraction (see Fig. 1) is an extraction technique in which a chemical is extracted from the aqueous phase in a hydrophobicity-dependent manner. In other words, more hydrophobic compounds are extracted more efficiently than are less hydrophobic compounds, similar to the bioconcentration process in biota. This condition can only be met by keeping the aqueous concentration essentially constant during the extraction or concentration process (see Fig. 1). The aqueous concentration will remain constant only if the amount of hydrophobic material into which a compound is partitioning is extremely small compared to the volume of the aqueous phase. This extraction procedure has the same characteristics as solid-phase microextraction, which is also an equilibrium (and nonexhaustive) partitioning procedure, in contrast to a quantitative extraction [27]. A solid-phase extraction disk (Empore® disk), which is chemically bound C₁₈ embedded in a Teflon® matrix, was selected. The rationale for this choice was the fact that bioconcentration in biota is related to the hydrophobicity of organic chemicals [28,29] and that partitioning onto C₁₈ is a good measure for hydrophobicity [30–32]. In addition, the use of the Empore disk allows us to add a very small volume of a C₁₈ solid phase to a large volume of water. As shown in our previous work, this procedure results in almost constant aqueous concentrations during the extraction [25]. The biomimetic approach for concentrating chemicals on a hydrophobic phase resembles extraction techniques using semipermeable membranes or similar devices, which have been used by Södergren [33] and Huckins and coworkers [34,35]).

Table 1. No-effect body residues at three different endpoints

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>No-effect body residues (mmol/kg lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality (fish)</td>
<td>25</td>
</tr>
<tr>
<td>Sublethal effects (fish)</td>
<td>5.0</td>
</tr>
<tr>
<td>Ecosystem-level effects (HC₅)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Endpoints used for calculating no-effect body residues are given here on a molar and lipid weight basis. In a risk assessment, we may compare the experimental data for body residues of individual compounds (BR) or for mixtures of chemicals, here defined as total body residues (TBRs), with these no-effect body residues.

TBRs has been developed, not by focusing on individual chemicals but by quantifying total molar concentrations of mixtures in one measurement. Results of this basic research have been published by Verhaar et al. [25] and Van Loon et al. [26].

The no-effect body residues for mortality is about a factor of two lower than the lethal body residue (= 50 mmol/kg).

Fig. 1. The principle of a biomimetic extraction procedure and estimation of total body residues (TBRs). In a biomimetic extraction, the aqueous concentration ($C_a$) remains constant during the extraction (a) and therefore concentrations of more hydrophobic chemicals will be higher on a solid ($C_b$) (b). Chemicals are extracted using a small amount of solid phase, containing $C_{18}$ (c) and the total molar concentration on this hydrophobic phase is determined and used as an estimate of the TBR (d).

Total body residues in biota can be estimated from measured total concentrations on the Empore disk ($\sum C_{\text{Empore disk}}$) using the equation below, an equation that was derived by Verhaar et al. [25] for a heterogeneous set of nonionic compounds.

$$TBR_{\text{est}} = 0.20 \sum C_{\text{Empore disk}}$$

in which $TBR_{\text{est}}$ is the estimated sum of body residues of individual chemicals ($\sum BR$). The equation was calculated based on the assumption that bioconcentration factors of non-polar organic chemicals are linearly related to octanol–water partition coefficients ($K_{\text{ow}}$) and on a linear relationship between partition coefficients onto the Empore disk and $K_{\text{ow}}$ as established by Verhaar et al. [25].

Determination of total molar concentrations. The measurement of total molar concentrations is a relatively new type of analysis [25,26]. Procedures for measuring total molar concentrations should, in principle, fulfill the following two conditions: the responses of individual chemicals must be equal, and the responses of individual chemicals in a mixture must be additive. We tested two analytical techniques: vapor pressure osmometry and gas chromatography–mass spectrometry (GC-MS) (total ion current). Both techniques are, within some limitations, well able to quantify total molar concentrations of organic compounds and analytical details can be found in [25] and [26].

Overview of experiments

An extensive description of the technique was given in earlier publications by Verhaar et al. [25] and Van Loon et al. [26]. In this paper we present the results of the application of...
this new method to 17 effluents and 13 surface water samples, covering a wide range of water types and chemical compositions. Total body residues were estimated for all these water samples and the results are interpreted from a toxicologic point of view.

MATERIALS AND METHODS

Sampling

Effluent samples (2 × 10 L; n = 17) were provided in spring 1995 by the Institute for Inland Water Management and Waste Water Treatment (RIZA), Lelystad, The Netherlands. The effluents, the sources of which are kept anonymous in this paper, were sampled from chemical industries (n = 8), paper industries (n = 2), metal industries (n = 2), a pesticide industry (n = 1), and sewage treatment plants (n = 3). These sampling locations cover many types of industrial effluents encountered in The Netherlands. Surface water samples (2 × 10 L, n = 12) were taken in spring 1995 from the River Rhine (Lobith, The Netherlands [NL]), the River Meuse at Eijsden (NL) and Luik (Belgium [B]), the River Eem (NL), the River Drentsche Aa (NL), the River Scheldt (B), Lake IJsselmeer (NL), Lake Ketelmeer (NL), Lake Markermeer (NL), the North Sea at Scheveningen (NL), the Wadden Sea at Pieterburen (NL), and the Westerscheldt estuary at Kruiningen (NL). These sampling locations cover most surface water types encountered in The Netherlands. Drinking water was sampled at the water works at Amsterdam (NL). All water samples were preserved by the addition of 5 ml of a silver nitrate solution (1 mg/L) to a 10-L water sample immediately after sampling, and were processed immediately at our laboratory.

Materials

Empore disks (C_{18} solid phase; 47-mm diameter; 90% C_{18}, 10% Teflon fibers) were purchased from J.T. Baker (Deventer, The Netherlands). These disks were cleaned thoroughly [26] prior to use. Homemade (RITOX, Utrecht, The Netherlands) Empore disk holders were used. The vapor pressure osmometer used in this study consisted of a Model Osmomat 070 cell unit and a Model Osmomat 070/090 control unit (Gonotec, Berlin, Germany). A GC-MS combination from Carlo Erba Instrument (Milan, Italy), consisting of a Model OC516 on-column control unit, a Model MFC500 gas chromatograph, and a Model QMD-1000 quadrupole mass spectrometer, was used for total molar determinations. A GC-MS combination consisting of a HP 5890 series II (Palo Alto, CA, USA) gas chromatograph, coupled to a SSQ 710 quadrupole mass spectrometer (Finnigan-MAT, San Jose, CA, USA), was used for the identification and quantification of major bioaccumulable compounds.

Procedures

The biomimetic extraction and total molar determination procedures are described in detail elsewhere [25, 26]. Briefly, a biomimetic extraction was performed in a 10-L glass bottle by stirring (300 rpm) a 10-L water sample with a 13-mm-diameter (=40-mg) Empore disk in an Empore disk holder at 22°C under dark conditions. After 7 d, the water sample was refreshed and a total partitioning time of 14 d was allowed. The disk was then removed from the water sample, suspended matter was wiped off with a tissue, and the disk was inserted immediately into 2 ml of cyclohexane. Humic acids do not bind to the Empore disk in a significant amount and therefore do not disturb the procedure [26]. Following a 3-d extraction, the Empore disk was removed from cyclohexane and, if necessary, the extract was evaporated under nitrogen to approximately 200 μl. As shown in previous work, recoveries of extraction from the Empore disk are higher than 95% [26]. The volume of the concentrated extracts was determined gravimetrically; 100 μl was inserted into a syringe for immediate vapor pressure osmometry (VPO) determination and the remaining 100 μl was used for the GC-MS determination of total molar concentrations.

Total molar determinations using GC-MS were performed as follows. First, a GC-MS total molar determination was performed on the 2-ml cyclohexane extract to determine if concentration of the extract by evaporation to 200 μl was necessary. Then, the internal standard (2,4,5-trichlorotoluene; approx. 2 or 20 nmol) was added to the extract. The GC-MS conditions used were: injection volume, 5 μl; a retention gap (5 m long × 0.31-mm i.d.; J&W, Folsom, CA, USA); a short column (phase, DB-1; 5-m long × 0.32-mm i.d.; film thickness, 0.1 μ; J&W); a rapid temperature program 40°C (2 min) to 290°C (0 min) at 30°C/min; mass spectrometry scan range, m/z 34 to 500; cycle time, 0.5 s; duplicate determinations were performed. Total molar determinations using VPO were performed as follows. A cell temperature of 37°C and a signal stabilization time of 8 min were used. Measurements were performed in triplicate.

Identification and quantification were carried out for major compounds in the effluent extracts. The GC-MS conditions used were: column, CP-Sil-5-CB (50-m long × 0.25-mm i.d.; film thickness, 0.5 μm; Chrompack, Middleburg, The Netherlands); injection mode, splitless; injection volume, 2 μl; temperature program, 75°C (2 min) to 300°C (10 min) at 5°C/min; ion source temperature, 150°C; electron impact ionization; electron energy, 70 eV; emission current, 400 μA; scan range, m/z 35 to 450; cycle time, 0.6 s: solvent delay time, 6.0 min. Quantitative results were calculated from the compounds total ion current (TIC) and the internal standard (2,4,5-trichlorotoluene) TIC. Total molar concentrations in cyclohexane, in the Empore disk, and in the lipid phase of aquatic organisms were calculated using equations derived and reported elsewhere [25,26].

RESULTS AND DISCUSSION

Total bioaccumulation in effluents and surface water samples

The results of the experiments for surface waters and effluents are given in Tables 2 and 3, respectively. These tables show the TBR_{m,s}, which are all calculated based on the measured concentrations on the Empore disk and on the equation. The estimated body residues reflect the concentration at the time of sampling.

For almost all effluent samples, total molar concentrations could be measured via both osmometry and GC-MS, whereas osmometry was not sensitive enough for most of the surface water samples. The data for effluents, obtained via osmometry and GC-MS, are compared in Figure 2. Although the principles of these techniques are completely different, the measured data compare quite well. The maximum difference is a factor of about two, which is not negligible but which is acceptable for risk assessment purposes. The advantage of VPO is that it actually determines total molar concentrations more accurately, but it is less sensitive and it underestimates the concentrations of more volatile chemicals. Gas chromatography–mass spectrometry is more sensitive, but the molar response factors
Estimating total body residues of complex mixtures

**Table 2. Estimated total body residues (TBR<sub>est</sub>) and dissolved organic carbon (DOC) contents of surface water samples and a drinking water sample**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Surface water location</th>
<th>TBR&lt;sub&gt;est&lt;/sub&gt;: Vapor pressure osmometry (mmol/kg)</th>
<th>TBR&lt;sub&gt;est&lt;/sub&gt;: Gas chromatography±mass spectrometry (mmol/kg)</th>
<th>DOC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>River Eem, Baarn</td>
<td>1.26</td>
<td>1.51</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>River Drentsche Aa</td>
<td>&lt;0.61</td>
<td>0.243</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Lake Ketelmeer</td>
<td>&lt;0.61</td>
<td>0.336</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>Lake Markermeer</td>
<td>&lt;0.61</td>
<td>0.174</td>
<td>8.7</td>
</tr>
<tr>
<td>5</td>
<td>North Sea, Scheveningen</td>
<td>&lt;0.61</td>
<td>0.262</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>River Rhine, Lobith</td>
<td>&lt;0.61</td>
<td>0.347</td>
<td>3.6</td>
</tr>
<tr>
<td>7</td>
<td>River Rhine, Eijdsen</td>
<td>&lt;0.61</td>
<td>0.334</td>
<td>3.6</td>
</tr>
<tr>
<td>8</td>
<td>River Meuse, Eijdsen</td>
<td>&lt;0.61</td>
<td>1.025</td>
<td>3.7</td>
</tr>
<tr>
<td>9</td>
<td>River Meuse, Luik</td>
<td>&lt;0.61</td>
<td>1.36</td>
<td>3.7</td>
</tr>
<tr>
<td>10</td>
<td>River Scheldt, Antwerp</td>
<td>3.08</td>
<td>2.65</td>
<td>4.2</td>
</tr>
<tr>
<td>11</td>
<td>River Meuse, Eijdsen</td>
<td>1.61</td>
<td>0.959</td>
<td>3.7</td>
</tr>
<tr>
<td>12</td>
<td>Westerscheldt estuary, Kruiningen</td>
<td>&lt;0.61</td>
<td>0.467</td>
<td>1.2</td>
</tr>
<tr>
<td>13</td>
<td>Wadden Sea, Pieterburen</td>
<td>&lt;0.61</td>
<td>0.069</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
<td>Lake IJselmeer</td>
<td>0.61</td>
<td>0.089</td>
<td>6.2</td>
</tr>
<tr>
<td>15</td>
<td>Drinking water, Amsterdam</td>
<td>&lt;0.61</td>
<td>&lt;0.045</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*VPO = vapor pressure osmometry; GC-MS = gas chromatography±mass spectrometry.

**Table 3. Estimated total body residues (TBR<sub>est</sub>) and dissolved organic carbon (DOC) contents of effluent samples**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>TBR&lt;sub&gt;est&lt;/sub&gt;: Vapor pressure osmometry (mmol/kg)</th>
<th>TBR&lt;sub&gt;est&lt;/sub&gt;: Gas chromatography±mass spectrometry (mmol/kg)</th>
<th>DOC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110</td>
<td>168</td>
<td>280</td>
</tr>
<tr>
<td>2</td>
<td>13.5</td>
<td>23.5</td>
<td>116</td>
</tr>
<tr>
<td>3</td>
<td>&lt;0.61</td>
<td>0.39</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>60.7</td>
<td>35.7</td>
<td>225</td>
</tr>
<tr>
<td>5</td>
<td>3.02</td>
<td>5.49</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>70.6</td>
<td>55</td>
<td>122</td>
</tr>
<tr>
<td>7</td>
<td>&lt;0.61</td>
<td>0.79</td>
<td>5.9</td>
</tr>
<tr>
<td>8</td>
<td>&lt;0.61</td>
<td>1.4</td>
<td>7.8</td>
</tr>
<tr>
<td>9</td>
<td>1.92</td>
<td>2.68</td>
<td>4.4</td>
</tr>
<tr>
<td>10</td>
<td>2.31</td>
<td>5.4</td>
<td>56</td>
</tr>
<tr>
<td>11</td>
<td>3.53</td>
<td>4.29</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>6.54</td>
<td>9.9</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>8.08</td>
<td>11.7</td>
<td>18</td>
</tr>
<tr>
<td>14</td>
<td>39.4</td>
<td>30</td>
<td>175</td>
</tr>
<tr>
<td>15</td>
<td>12.3</td>
<td>16</td>
<td>46</td>
</tr>
<tr>
<td>16</td>
<td>2.16</td>
<td>2.75</td>
<td>13</td>
</tr>
<tr>
<td>17</td>
<td>41.4</td>
<td>47.3</td>
<td>45</td>
</tr>
</tbody>
</table>

*VPO = vapor pressure osmometry; GC-MS = gas chromatography±mass spectrometry.

**Toxicologic interpretation of TBR<sub>est</sub> values**

How can these values for TBR<sub>est</sub> be interpreted? In fact, the TBR<sub>est</sub> parameter is a screening tool for the total bioaccumulation of organic chemicals in biota. Clearly, this is relevant information but bioaccumulation is not a toxicologic endpoint. The measured values for TBR<sub>est</sub> can be compared with no-effect body burdens for baseline toxicity (see Table 1). Such a comparison is shown in Figures 3 and 4 where, in addition to the TBR<sub>est</sub> values, no-effect body burdens for three endpoints are given. The following conclusions can be drawn assuming that all chemicals act by baseline toxicity: some of the effluent samples (samples 1, 4, 6, 14, and 17) will cause severe effects because the TBR<sub>est</sub> values are above the no-effect body residue level for mortality of 25 mmol/kg, whereas several other effluents may cause sublethal effects because TBR<sub>est</sub> values exceed the critical level of 5.0 mmol/kg.

The levels in surface waters are all below the no-effect level for sublethal fish toxicity, although the River Scheldt and the River Meuse (samples 8–10) contain levels close to this level. Only the Wadden Sea (sample 13) and Lake IJselmeer (sample 14) contain concentrations below the level for ecosystem effects (0.25 mmol/kg).

The assumption that all chemicals only have baseline tox-
correspond to those in Table 2). The TBRest is calculated using vapor pressure osmometry (VPO) measurements; gas chromatography–mass spectrometry (GC-MS) results are used in those cases where the VPO results are below the detection limit.

Fig. 3. Estimated total body residues (TBRest) of several surface waters and drinking water in The Netherlands and Belgium (numbers correspond to those in Table 3). The TBRest is calculated using vapor pressure osmometry (VPO) measurements; gas chromatography–mass spectrometry (GC-MS) results are used in those cases where the VPO results are below the detection limit.

In many cases, chemicals with more specific modes of action (such as many pesticides) are present. Aqueous effect concentrations or effect body residues of such compounds are usually much lower than those for baseline toxicity chemicals. On the other hand, if the actual aqueous concentration or body residue of a chemical with a specific mode of action is below the effect level for specific toxicity, this same chemical will still contribute to the overall baseline toxicity [18]. If many chemicals are present, these small contributions may lead to a relatively high contribution to the overall baseline toxicity because baseline toxicity is completely additive [18].

Additional information from GC-MS chromatograms

Gas chromatography–mass spectrometry was used to measure total molar concentrations and a minimal separation of individual compounds was achieved by using a very short column and a high temperature programing rate. However, in spite of the relatively low resolution, the GC-MS runs for the individual samples still contain relevant information and examples are presented in Figure 5 for a few effluent and surface water samples. Results from a blank sample (clean Empore disk) are shown in Figure 5 as well. Effluent sample 3 contains only one or a few components, whereas effluent sample 5 contains a much larger number of compounds. Surface water samples from the River Scheldt (sample 10) contain relatively high concentrations of a wide range of different compounds, whereas the River Rhine water sample (sample 6) contains a few components at high concentrations and many others at relatively low concentrations. Lake IJsselmeer (surface water sample 14) is an example of a relatively clean water system containing several compounds at low concentrations.

These chromatograms may give some additional and relevant information that may guide more detailed analyses. For example, if one or only a few components contribute to a large extent to the TBRest, a more detailed analysis and actions should focus on these components. On the other hand, if many different compounds contribute to the TBRest, general measures should be taken to reduce the load of chemical pollutants.

The effluent samples were also analyzed in detail using GC-MS. The results of these detailed analyses are given in the Appendix, together with values for the $K_{ow}$ only those compounds that contribute more than 10% to the TBRest are listed. It is interesting to note that the major contributions in most cases come from moderately to highly hydrophobic (log $K_{ow} > 4$) chemicals. Because the Empore disk extraction as described in this paper is a true partition extraction, the more relevant hydrophobic chemicals become more apparent in the analysis; this is certainly an advantage over exhaustive extractions.

Relation between TBR and dissolved organic carbon content

The group parameter presented here is new and a comparison with established group parameters is therefore of interest. We also obtained data for the dissolved organic carbon (DOC) concentrations for all samples. Comparisons between these DOC concentrations and TBRest for the surface water and effluent samples are given in Figures 6 and 7, respectively. We did not expect a significant correlation because the two procedures measure completely different properties. The DOC content gives information on the total content of dissolved organic compounds in the aqueous phase, whereas the parameter TBRest gives information on total concentrations in biomimetic extracts from the aqueous phase in which more hydrophobic compounds will contribute more strongly than less hydrophobic ones.

It was somehow a surprise that the correlation between the DOC content and TBRest was significant for the effluents (Fig. 7). Such a relation may indicate that the chemical structures and hydrophobicity of the compounds present in effluents are not extremely different from one sample to another.

There is no correlation between the DOC contents and TBRest for surface waters (Fig. 6). This is not unexpected; surface waters generally contain high concentrations of humic substances and these natural compounds will contribute to a large extent to the measured DOC concentrations. Such natural occurring compounds are hydrophilic and will not be concentrated on the Empore disk [26] and, therefore, do not contribute to the TBRest. A good example is the River Drentsche Aa, which contains a high DOC concentration but the TBRest has one of the lowest values. An additional advantage of the parameter is that it will only detect the bioavailable fraction of nonionic organic micropollutants (mostly of anthropogenic origin) and will not be influenced by high levels of naturally
Estimating total body residues of complex mixtures

Fig. 5. Gas chromatography–mass spectrometry chromatograms for a few surface water and effluent samples concentrated on the Empore disk (sample numbers correspond with those in Tables 2 and 3).

occurring and nontoxic carbon- or halogen-containing macromolecules. In future research, we will compare the TBR parameter with other standard group parameters such as AOX. It is well known that a large fraction of the AOX is related to nontoxic humic acid substances [37].

CONCLUSIONS

The parameter TBR\textsubscript{est} gives information on the total bioaccumulation and bioavailability of mixtures of chemicals in the aqueous phase. Information on TBR\textsubscript{est} is useful for getting an impression of the total load of organic chemicals in aquatic organisms in a toxicologically relevant manner. The fact that TBR\textsubscript{est} magnifies chemicals with a high potential for bioaccumulation is an important advantage. Because total concentrations instead of individual compounds are determined, the outcome also includes those chemicals that are usually not measured because they cannot be identified or because their concentrations are below individual detection limits.

In addition to being a parameter for the bioaccumulation of mixtures, TBR\textsubscript{est} is also a measure for the total residues of chemicals with baseline toxicity, including the contributions of chemicals with specific modes of action to this overall baseline toxicity. In that sense, TBR\textsubscript{est} is also a toxicologic parameter. If the total residues exceed a certain effect level, there is reason for concern. However, if the residues are below the critical effect levels, effects cannot be ruled out because chemicals with more specific modes of action may be present.

The advantage of working with body residues is that for chemicals (and chemical mixtures) with only baseline toxicity, the no-effect body residue is constant for a certain endpoint. Because of this, the effects of mixtures can be evaluated by using the equation: TBR/no-effect body residue < 1.0, in which TBR is a measurable quantity. The evaluation of mixture effects based on aqueous concentrations is based on the equation \(\Sigma \{\text{PEC}/\text{PNEC}\} < 1.0\), where PEC is the predicted environmental concentration, and PNEC is the predicted no-effect concentration; this equation can only be used if the concentrations of all individual chemicals are known.

Fig. 6. Relationship between estimated total body residues (TBR\textsubscript{est}) and dissolved organic carbon (DOC) content for surface water samples (data are from Table 2).

Fig. 7. Relationship between estimated total body residues (TBR\textsubscript{est}) and dissolved organic carbon (DOC) content for effluent samples (data are from Table 3).
The new parameter TBR_{aq} also has its limitations. Total body residues are measured on a biomimetic hydrophobic phase as a surrogate for biota. Properties other than hydrophobicity can influence bioaccumulation in biota. For example, the molecular size of a chemical may decrease uptake [38,39], and chemicals that are biotransformed relatively rapidly will have lower bioconcentration factors than predicted by their hydrophobicity alone [40,41]. Uptake of very hydrophobic compounds may also take place via routes other than simply by diffusion; in those cases ingestion of food or sediment particles may become the predominant routes for uptake [42–44]. Bearing in mind these limitations, the results from this procedure can be interpreted properly.

This manuscript describes one of the first applications of the concept of measuring TBRs. We believe that this concept is very useful. It is one of the first procedures that estimates the total toxicity of a certain toxicologic compound class based on chemical analysis; this concept may be a pattern for handling other toxicologic groups of compounds. As with all new procedures, this new method should be proven in further applications.

Acknowledgement—The research described in this manuscript was supported financially by the Institute for Inland Water Management and Waste Water Treatment, Lelystad, Netherlands; the Directorate-General for Environmental Protection, The Hague, The Netherlands; and by the European Commission, Directorate General for the Environment.

REFERENCES


### APPENDIX

Major organic micropollutants as identified in biomimetic extracts from effluents

<table>
<thead>
<tr>
<th>Compound name</th>
<th>log $K_{ow}$</th>
<th>Application</th>
<th>Effluent type</th>
<th>% Of TBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>tert-Butyl-2-methoxyphenol isomer</td>
<td>3.15</td>
<td>Antioxidant</td>
<td>Polymer industry</td>
<td>52</td>
</tr>
<tr>
<td>4-Chlorophenyl-2,4,5-trichlorophenyl sulphone</td>
<td>4.94</td>
<td>Nonsystematic acaricide</td>
<td>Chemical industry</td>
<td>21</td>
</tr>
<tr>
<td>2,6-Diisobutylphenol</td>
<td>5.39</td>
<td>Antioxidant, intermediate</td>
<td>Chemical industry</td>
<td>11</td>
</tr>
<tr>
<td>Di(isobutyl)phenol isomers</td>
<td>5.39</td>
<td>Antioxidant</td>
<td>Metal industry</td>
<td>10</td>
</tr>
<tr>
<td>Diisobutylphthalate</td>
<td>4.39</td>
<td>Plasticizer</td>
<td>Chemical industry</td>
<td>61</td>
</tr>
<tr>
<td>Dichlorobenzonitrile isomer</td>
<td>3.00</td>
<td>Unknown</td>
<td>Paper industry</td>
<td>12</td>
</tr>
<tr>
<td>2,4-Dichloro-1-methylbenzene</td>
<td>4.07</td>
<td>Intermediate product</td>
<td>Chemical industry</td>
<td>13</td>
</tr>
<tr>
<td>Di(2-propyl)phenol isomers</td>
<td>4.33</td>
<td>Plasticizer, surfactant, intermediate</td>
<td>Metal industry</td>
<td>56</td>
</tr>
<tr>
<td>HHCB</td>
<td>6.06</td>
<td>Fragrance in washing agents</td>
<td>Sewage</td>
<td>46</td>
</tr>
<tr>
<td>1-(Hydroxymethyl)methylamino-adamantane</td>
<td>2.61</td>
<td>Unknown</td>
<td>Pesticide industry</td>
<td>48</td>
</tr>
<tr>
<td>Limonene</td>
<td>4.35</td>
<td>Flavor, fragrance</td>
<td>Sewage</td>
<td>33</td>
</tr>
<tr>
<td>9-Octadecenoic acid 2,3-dihydroxypropylester</td>
<td>NA</td>
<td>Drying oil</td>
<td>Metal industry</td>
<td>13</td>
</tr>
<tr>
<td>Terphenyl isomer</td>
<td>5.9</td>
<td>Unknown</td>
<td>Chemical industry</td>
<td>13</td>
</tr>
<tr>
<td>AHTN</td>
<td>6.25</td>
<td>Fragrance in washing agents</td>
<td>Sewage</td>
<td>19</td>
</tr>
<tr>
<td>Triphenyl phosphine sulfide</td>
<td>NA</td>
<td>Rubber production</td>
<td>Pesticide industry</td>
<td>16</td>
</tr>
</tbody>
</table>

$K_{ow}$ = octanol–water partition coefficient. All log $K_{ow}$ values are calculated using the CLOGP program from Daylight Information Systems, Irvine, California, USA. TBR = total body residue.

* Calculated with the tert-butyl group in meta position.
* Calculated as the 2,6 isomer.
* Calculated as the 1,2 isomer.
* Calculated as the 3,5 isomer.
* HHCB = 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethycyclopenta-gamma-2-benzopyran.
* NA = not available (impossible to calculate due to missing fragment value).
* AHTN = 6-acetyl-1,1,2,4,4,7-hexamethyltetraline.