A study on the behaviour of pesticides and their transformation products in the Scheldt estuary using liquid chromatography-electrospray tandem mass spectrometry

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Off-line solid-phase extraction (SPE) combined with liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS-MS) was used to study the estuarine behaviour of the polar pesticides, atrazine, chloridazon, diuron and metolachlor, and their transformation products (TPs), hydroxyatrazine (HA), desisopropylatrazine (DIA), desethylatrazine (DEA), 3,4-dichlorophenylmethylurea (DPMU) and monuron. The compounds were identified by comparing their LC retention times and product-ion spectra with those of standard solutions. In all but one case the detection limits of the method were sufficient to determine the compounds of interest over the entire salinity range in the estuary. The concentrations of the dissolved pesticides ranged from 70 ng l^{-1} for chloridazon to 1350 ng l^{-1} for diuron. The levels of TPs were 3–8% of the levels of their parent pesticide. The mixing plots of polar pesticides and their TPs indicated that TPs, which are present in fresh river water, are conservatively transported to the sea and that no additional amounts of TPs are formed during their transport through the estuary. The one exception was HA, of which approximately 10% of the amount transported to the North Sea is formed in the lower part of the estuary by photochemical oxidation of atrazine. The latter was concluded from the ratios of each analyte over the sum total of the parent pesticide and all TPs along the salinity gradient, which proved to be a useful tool for identifying such estuarine transformations.

Introduction

A broad range of modern, *i.e.*, relatively polar, pesticides are used in agriculture. In the last decade, many studies have shown that enhanced concentrations of these pesticides are frequently encountered in inland water, groundwater and drinking water. Considerably less work has been carried out with respect to the marine environment. For a proper risk assessment of modern pesticides, information is required on their occurrence and behaviour in the marine environment. In earlier studies we determined the time profiles of the concentrations of dissolved pesticides in the freshwater sources discharging into the Scheldt estuary.[1] Next to parent compounds such as atrazine, simazine, alachlor and metolachlor, relatively large amounts of the atrazine TP, DEA, were found to be transported into the estuary. To assess the overall risk caused by pesticides, it is, therefore, important to include these TPs in environmental monitoring programmes. This is even more true because over 50% of the TPs of triazines, carbamates and phenoxypropionic acids seem to pose a similar or even higher risk than their parent pesticides.[2] Several studies have reported the presence of TPs in rivers that discharge into the marine environment.[3–5] For the Scheldt, we demonstrated that low ng l^{-1} levels of the TPs of diuron and atrazine were transported into its estuary.[6] In another study, mixing plots, which describe the relation between dissolved concentration and salinity in an estuary, revealed non-conservative behaviour, *i.e.*, estuarine losses, for pesticides such as simazine, dichlorvos and diazinon.[1,7] In some cases, this was attributed to estuarine degradation, *i.e.*, to the possible formation of TPs in the estuary.[1,7]

A reversed-phase LC separation method was combined with electrospray tandem mass spectrometry (LC-ESI-MS-MS), to enable the quantification of TPs and, also, of relatively polar pesticides such as diuron and chloridazon. It was successful for the analysis of pesticides and TPs in surface and estuarine water samples down to the low ng l^{-1} level.[6] Tandem mass spectrometry was used because of its high selectivity and its increased potential in compound identification. Off-line SPE with LC-ESI-MS-MS was used to achieve the analyte detectability required for the compounds of interest. However, whereas in earlier work we focussed our attention on the estuarine behaviour and net fluxes of parent pesticides, in the present study both parent pesticides and their TPs have been studied.

Experimental

Reagents

Diuron, atrazine, DEA, DIA, HA, anthranilisopropylamid (AIPA), chloridazon, and metolachlor were obtained from Riedel-de Haën (Seelze, Germany) and DMPU from Dr. Ehrenstorfer (Augsburg, Germany). Standards were at least 98% pure. Stock standard solutions were prepared by dissolving 10 mg in 10 ml of methanol and were stored in the dark at −20 °C. For HA, a few drops of concentrated formic acid (J.T. Baker, Deventer, The Netherlands) were added to promote dissolution. HPLC gradient grade water and methanol were obtained from J.T. Baker. Nitrogen (99.999% purity) and argon (99.9995% purity) were from Praxair (Oevel, Belgium).

Sample preparation

Sample preparation and analyses were performed according to a method developed earlier.[6] Sampling was performed from an

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oceanographic vessel using a pumping unit equipped with a salinity monitor, which allowed us to select samples at regular salinity intervals along the salinity gradient. Next to salinity, the temperature, oxygen content, scattering (turbidity), pH and natural fluorescence were monitored. Since the study is aimed at identifying pesticide losses during transport to the sea, a sampling procedure was developed which effects immediate preservation. Filtration and off-line SPE of water samples were performed onboard the sampling vessel followed by storage of the extraction cartridge at −20 °C until elution and subsequent analysis at the laboratory. Water samples (1 l) were filtered though a 0.45 μm ME-25 filter (Schleicher and Schuell, Dassel, Germany) to remove particulate matter. For the triazines, which are relatively polar compounds with log K_{ow} values ranging from 1.15 to 2.61 (see Table 1), it has been demonstrated that the amounts associated with the particulate phase are negligible.[10–12] With the exception of metolachlor, similar log K_{ow} values are reported for the other compounds. However, even for the moderately polar compound metolachlor it is estimated that the contribution of the particulate pesticide concentration is less than 10% of the total.[13] The LiChrolut EN cartridges (200 mg, Merck, Darmstadt, Germany) were conditioned with 5 ml of methanol and, next, 5 ml of HPLC grade water. After conditioning, the sample was added—care being taken that the cartridge did not run dry between the conditioning and the sample addition. The cartridges were eluted with 3 × 3 ml of methanol. 100 μl of an internal standard solution (1 ng μl^{-1} AIPA in methanol) was added to correct for variations in detector response between LC runs. The extracts were evaporated to a volume of approximately 200 μl under a gentle stream of nitrogen and 20 μl were injected on the LC column.

Chromatographic and mass spectral analysis

An HP1090 LC system equipped with a ternary solvent delivery unit (Hewlett-Packard, Waldbronn, Germany) was used. Separations were performed on a 250 mm × 4.6 mm id Vydac (Hesperia, CA, USA) column packed with 5 μm C18-bonded silica. Gradient elution was performed with an aqueous 10 mM ammonium acetate buffer pH 4.5 (A) and methanol (B) with A : B (90 : 10, v/v) for 1 min and, next, linearly to A : B (10 : 90) in 19 min. The column was operated at a flow rate of 1 ml min^{-1}, 70 μl min^{-1} being directed to the ESI interface *via* a post-column splitter. Tandem MS was performed on a VG Quattro II triple-stage quadrupole equipped with a dual electrospray/atmospheric pressure chemical ionisation (ESI/APCI) source (Micromass, Altrincham, UK). The source temperature was set at 80 °C, the ESI capillary voltage at 3.5 kV and the skimmer lens offset at 5 V. The standard dwell time was 1.5 s; it was adjusted to lower values if required because of the number of multiple reaction monitoring (MRM) scans per segment (see below). Nitrogen was used as both the drying and nebulizing gas at flow rates of 350 l h^{-1} and 15 l h^{-1}, respectively. The argon pressure in the collision cell was 2.5 μbar.

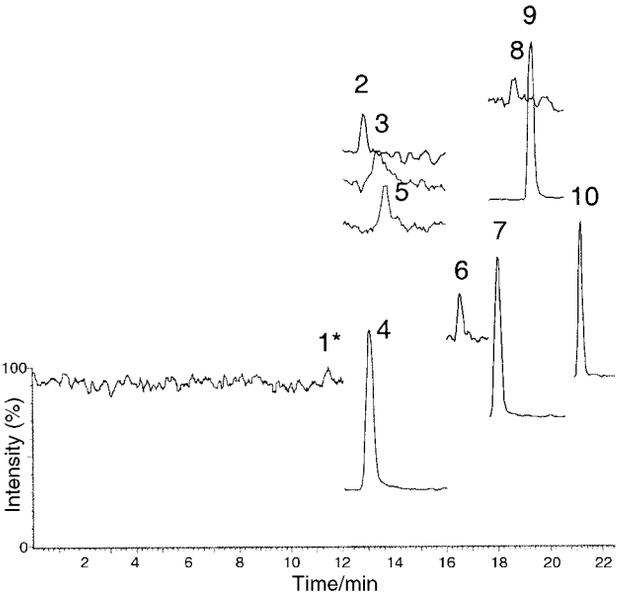

Fig. 1 LC-ESI-MS-MS of polar pesticides and their transformation products in the Scheldt estuary at the sea-end station (salinity 33‰). Peak assignment: 1, desisopropylatrazine (below detection limit); 2, chloridazon; 3, hydroxyatrazine; 4, AIPA (internal standard); 5, desethylatrazine; 6, monuron; 7, atrazine; 8, DPMU; 9, diuron; 10, metolachlor.

Results and discussion

Analysis and identification

The LC-ESI-MS-MS analyses were performed according to a method developed earlier where cone voltages and collision energies were optimized for maximum sensitivity.[6] In Fig. 1 the chromatograms for the most seaward sample (salinity 31‰) is shown. All compounds, except DIA, could be determined over the full salinity range although in some cases the concentrations found in the most seaward sample were rather close to the detection limits. Unfortunately, these limits of detection were somewhat poorer than in our earlier studies, which probably reflects some loss of performance of the analytical set-up. The precision was, however, fully satisfactory, with method relative standard deviations (RSDs) of 3–6% ($n = 3$; samples spiked at 40 ng l^{-1}).

It is generally agreed that one parent–product ion combination is insufficient for the identification of target compounds. Therefore, product ion spectra were recorded and compared with spectra recorded with standard solutions (Fig. 2). Identification was performed for the lower salinity samples because they contained the higher pesticide concentrations. As

Table 1 Detection limits and physical chemical characteristics of pesticides and TPs

Compound	DL[a]/ng l^{-1}	DT_{50}[b] water : sediment/d	log K_{ow}	log K_{oc}	H[c]/Pa m^3 mol^{-1}
Atrazine	0.7	85 (ref. 9)	2.61 (ref. 9)	2.1 (ref. 9)	$2.90e^{-4}$ (ref. 9)
DIA	6		1.15 (ref. 2)		
HA	8		2.43 (ref. 2)		
DEA	2	72 (ref. 9)	1.51 (ref. 9)	1.6 (ref. 9)	
Diuron	2		2.68 (ref. 9)	2.4 (ref. 9)	$1.05e^{-9}$[d] (ref. 9)
Monuron	2		3.05 (ref. 10)		
DPMU	6		—		
Chloridazon	7	72[e] (ref. 9)	1.14 (ref. 9)	2.08 (ref. 9)	$3.68e^{-3}$ (ref. 9)
Metolachlor	0.3	>120 (ref. 9)	3.68 (ref. 9)	2.30 (ref. 11)	$2.20e^{-3}$ (ref. 8)

[a]DL = detection limit; $S/N = 3$ in real samples. [b]DT_{50} = degradation half-life. [c]H = Henry's law constant. [d]Calculated with $H = P/Pa\ S^{-1}$ (mol m^{-3}). [e]Hydrolysis at pH 7; 114 d at pH 5 and 98 d at pH 9.

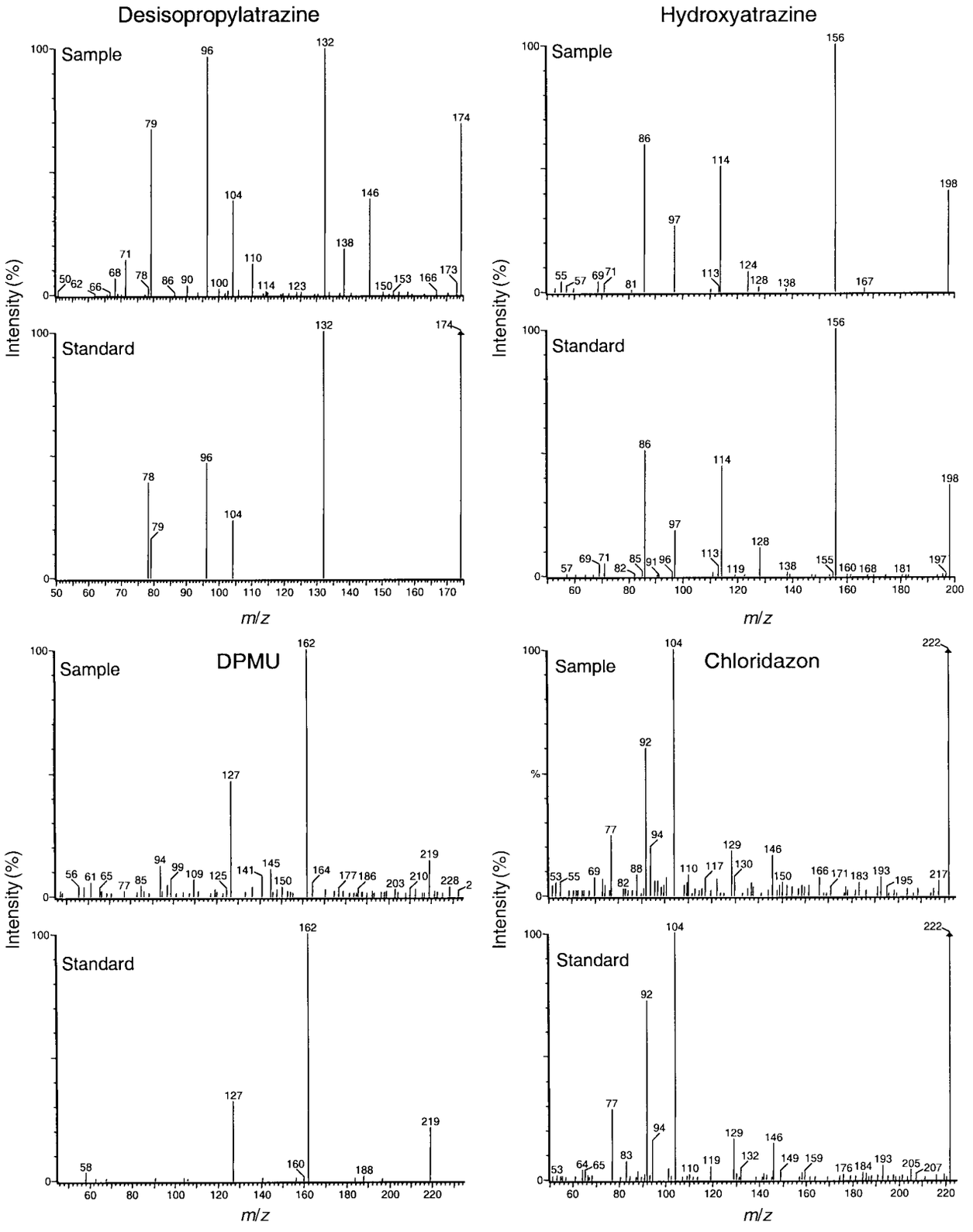

Fig. 2 Product-ion spectra of four selected target analytes (DEA, HA, DPMU and chloridazon). Spectra of the samples taken at the river-end station (salinity 1‰; top) and standards (bottom) are compared.

is illustrated by the four examples shown, good agreement between the standard and sample spectra was invariably obtained with respect to the masses of the product ions as well as their relative intensities.

Estuarine behaviour study

By constructing mixing plots, *i.e.*, establishing the relation between the dissolved concentrations of the pesticides and the salinity of the water samples, information is obtained on the chemical and physical fate of compounds within the estuary.[1,14] The mixing plots for the selected pesticides and their TPs are shown in Fig. 3. In general, concentrations are seen to decrease from the river-end member towards the open sea due to the mixing of the river water with the relatively uncontaminated seawater. In an earlier study we showed that unequivocal interpretation of the mixing plots requires knowledge of the history of pesticide concentrations in the freshwater sources

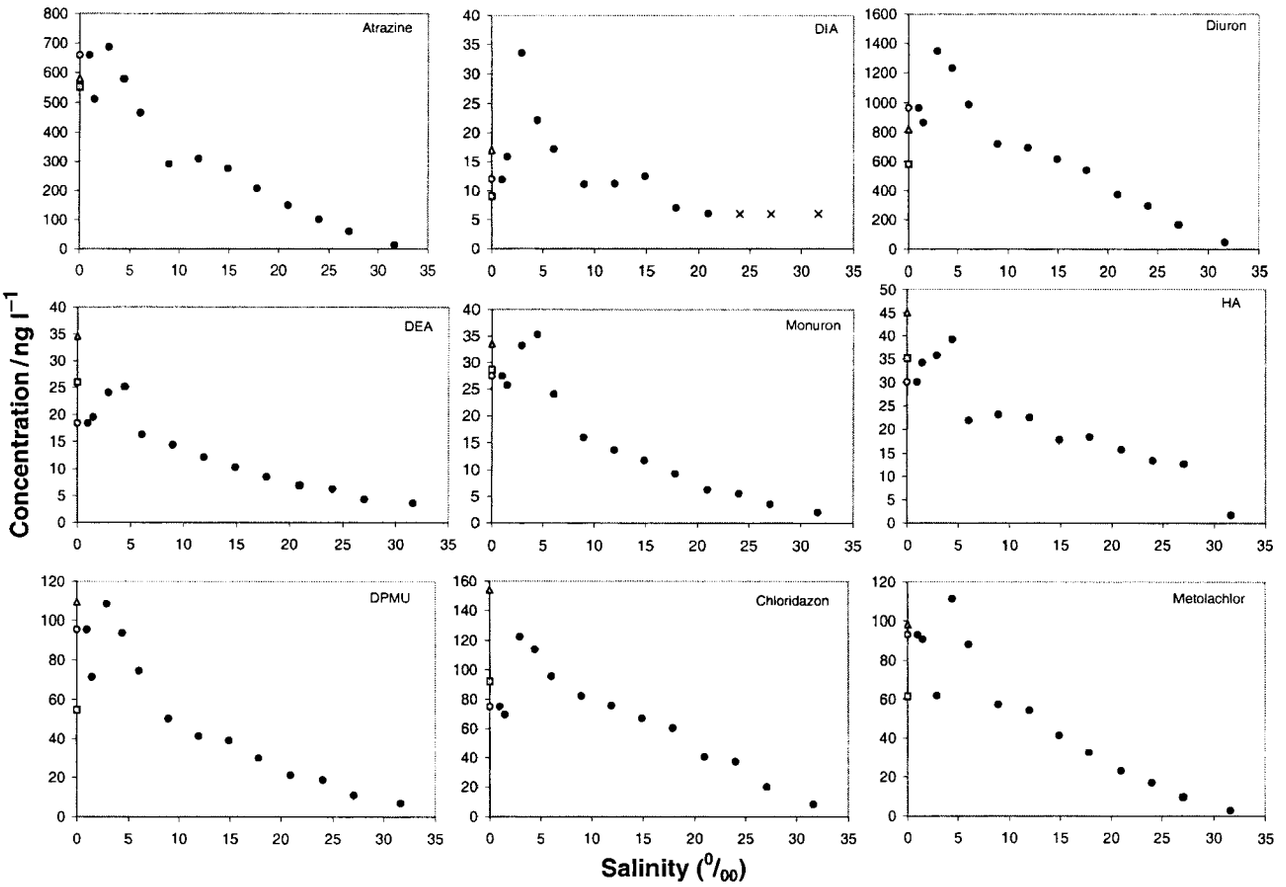

Fig. 3 Mixing plots of selected pesticides and their TPs in the Scheldt estuary. Pesticide concentrations in river-end member for □: June 11, 1998; △: June 26, 1998 and ○: July 27, 1998 are indicated on y-axis. Detection limits are indicated by a cross (×).

that discharge into the estuary.[1] Pesticide concentrations were, therefore, determined at the river-end member (salinity 1‰) starting 45 d prior to the sampling of the mixing plots, and are indicated on the y-axis of the mixing plots. To arrive at a conclusion on which observations in the mixing plot are related to the input data recorded during the 45 d window, we need to consider the timescale of water transport in the estuary, *i.e.*, the flushing time. In an earlier study we derived a relation between the freshwater discharge and the flushing time for the Scheldt estuary.[7] The freshwater discharge data are given in Fig. 4. In the period preceding the sample taking for the construction of the mixing plots (July 27 and 30, 1998), the freshwater discharge was relatively low and ranged between 43 and 137 $m^3 s^{-1}$. Under these conditions, the average flushing time of the estuary is 60 d.[7] The input data set comprised 45 d. Therefore, the observations at the most seaward stations should be related to input data prior to the 45 d window. From Fig. 3 it is clear that variations in pesticide input are reflected in fluctuations in the mixing plot in the low-salinity zone of the estuary. The fluctuations are less evident at higher salinities because the pesticides are increasingly dispersed as they are transported through the estuary. In the high-salinity zone, the mixing plots reveal an essentially linear relationship, which indicates conservative behaviour. It can be concluded, therefore, that the major part of the pesticides and TPs that are supplied by the river are transported to the North Sea, and that no significant transformation of these analytes takes place during that transport.

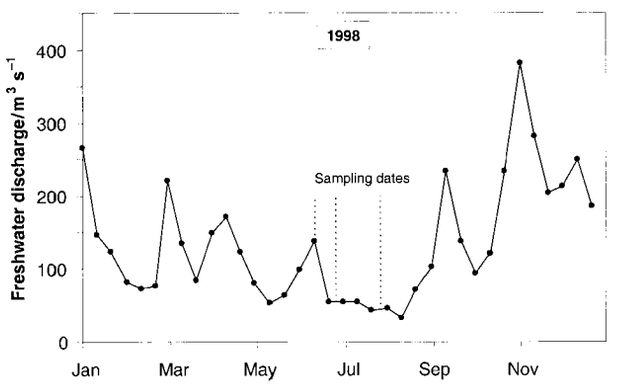

Fig. 4 10 d average freshwater discharges of the river Scheldt (1998).

The highest concentrations were found for the parent pesticides atrazine and diuron, *i.e.*, 680 and 1350 ng l^{-1}, respectively. The highest levels of their TPs were considerably lower, *i.e.*, 5, 4 and 6% of the level of atrazine for DIA, DEA and HA, respectively, and 3 and 8% of diuron for monuron and DPMU, respectively. For atrazine, HA is an important TP, which is in agreement with studies reported in literature.[15–18] The maximum concentrations of metolachlor and chloridazon were 110 and 150 ng l^{-1}, respectively.

Metolachlor was included in this study because earlier studies on its behaviour in the Scheldt estuary revealed a strong increase of metolachlor concentrations in the central part of the estuary. It was demonstrated that this was not caused by remobilization of metolachlor from sediment or suspended matter but that an additional and probably incidental direct emission source of metolachlor into the estuary, which was not related to its agricultural use, was responsible for the strong increase.[7] The mixing plot of metolachlor now reveals a linear mixing curve, which suggests conservative behaviour. These results confirm our earlier hypothesis of an incidental source. The relatively high degradation half-life (DT_{50}) of >120 d in water–sludge systems and low log K_{oc} value of 2.3 reported for

metolachlor agree with this finding (the physiochemical properties of the test compounds are summarized in Table 1). Chloridazon was included in this study because in the early 1990's its water quality objectives were frequently exceeded in the Scheldt.[19] Chloridazon was analysed by GC and, due to its relatively high polarity, rather poor chromatographic performance was reported even on a moderately polar GC column (which agrees with our own experience). The use of LC-ESI-MS-MS in this study allowed us to study the estuarine behaviour of chloridazon with a more robust method. The mixing plot of chloridazon also reveals a linear relationship, indicating conservative behaviour. This is in agreement with studies reported in the literature regarding the stability of chloridazon. The DT_{50} for hydrolysis at pH 7 is 72 d and no degradation was found in a water–sludge system after 56 d.[9] In addition, the reported log K_{oc} value of 2.08 and the relatively low Henry's law constant (H) of 3.7×10^{-3} (Pa m^3 mol^{-1}) strongly suggest that other estuarine loss processes like sorption and evaporation are absent.

Formation of TPs within the estuary

Fig. 3 shows that, in general, the highest river-end member concentrations were found in June (triangles on y-axis) and that, in the estuary, relatively low analyte concentrations for the first two (low-salinity) samples were followed by relatively high concentrations. This rather similar fluctuation pattern in analyte input implies that, if a pesticide and its TPs behave conservatively, the ratio between these compounds should remain relatively constant at any point of the estuary. In Fig. 5 the ratios of the concentrations of each analyte over the sum total of the parent pesticide and TPs are shown for atrazine and diuron. The ratios for the most seaward sample were discarded because of their relatively high imprecision caused by low TP concentrations which were often close to the detection limits (see Fig. 1). The ratio plots for diuron and its TPs show that there is no significant estuarine transformation of diuron. The TPs show a very slight decrease of the ratios towards the sea, which may be due to a lower persistence in estuarine waters than diuron. Unfortunately, there are no literature data to support this finding. For the TPs of atrazine, the ratios remained essentially constant in the low-salinity zone. At

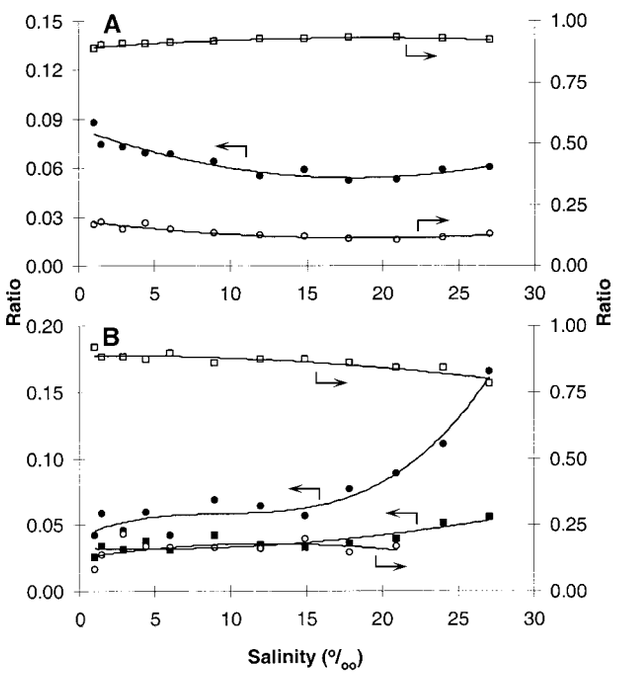

Fig. 5 Ratio of each analyte over the sum total of the parent pesticide and TPs for (A) monuron (○), DPMU (●) and diuron (□) and (B) DEA (■), HA (●), DIA (○) and atrazine (□).

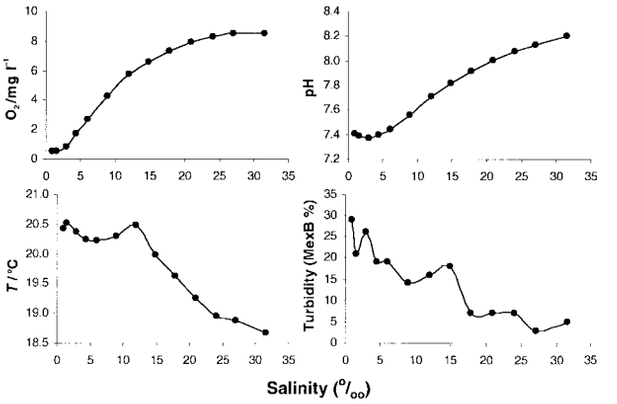

Fig. 6 General water quality parameters in the Scheldt estuary for July 1998.

higher salinities, however, the ratio for HA started to increase rather sharply, whereas the ratio of DEA increased only slightly. This may point to the transformation of atrazine into HA in the higher salinity zone. HA is formed through photochemical oxidation of atrazine.[15,16,18,20] The rates and pathways of photochemical transformations strongly depend on environmental parameters such as pH, oxygen content and ionic strength.[21] Some general water quality parameters, which were recorded during the sampling cruise, are shown in Fig. 6. The dissolved oxygen content is very low in the upper estuary and increases with salinity due to re-aeration and dilution with seawater. In addition, the low-salinity zone is highly turbid, which reduces the amount of light penetrating the water surface. Durand *et al.*[15] reported the enhanced photodegradation of atrazine in the presence of humic acids at slightly acidic pH. Owing to its riverine origin, the humic acid content will be higher in the upper estuary (0–20 km). Apparently, the higher oxygen content and lower turbidity prevail over the influence of the humic acids and pH and the photochemical transformation is stronger in the lower part of the estuary (70–105 km). DEA and DIA are mainly formed by microbiological dealkylation of atrazine in soil.[11,22] The results of Fig. 5 suggest that any contribution of microbiological transformation in the estuary, *e.g.*, by algae, is of minor importance compared to the pesticide concentrations already present in fresh river water.

Conclusions

Off-line SPE combined with LC-ES-MS-MS is a highly useful approach to determine the presence and behaviour of polar pesticides and their TPs down to the low ng l^{-1} level in estuaries. Compounds were identified by comparing their product-ion spectra and LC retention times with those of standard solutions. Riverine pesticide concentrations ranged from 70 ng l^{-1} for chloridazon to 1350 ng l^{-1} for diuron. The levels of TPs were considerably lower and ranged from 4 to 6% and from 3 to 8% of the levels of the parent pesticides atrazine and diuron, respectively. The mixing plots of polar pesticides and their TPs indicated that TPs, which are present in fresh river water, are conservatively transported to the sea and that no additional amounts of TPs are formed during their transport through the estuary. The one exception to this was HA; approximately 10% of the amount which is transported to the North Sea is formed in the lower part of the estuary.

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