Organic contaminants and trace metals in flounder liver and sediment from the Amsterdam and Rotterdam harbours and off the Dutch coast

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Organic contaminants [polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenylethers (PBDEs), nonylphenols], organotin compounds and trace metals (cadmium, chromium, mercury and zinc) were determined in flounder (*Platichthys flesus*) liver and sediment from the Amsterdam harbour (North Sea Canal) and Rotterdam harbour (Euromonding) and off the Dutch coast between the Amsterdam and Rotterdam harbour mouths in order to assess the level of contamination in these harbours and to study contamination gradients.

The highest concentrations of PCBs, several OCPs and PBDEs were found in the central areas of the Rotterdam and Amsterdam harbours. Compared with literature data, the PCB contamination in flounder liver may be considered to be relatively high. Nonylphenol concentrations were below the detection limit (0.15 mg kg^{-1} wet weight). Trace metal concentrations in flounder liver were generally low. Slightly elevated concentrations of cadmium and mercury were found in harbour sediments. Tributyltin (TBT) concentrations in sediments from the two harbour areas were higher than those from sediments of the two reference locations. Elevated polycyclic aromatic hydrocarbon (PAH) concentrations were found in sediments of the entire North Sea Canal area, including Amsterdam harbour. These concentrations were higher than those in the Rotterdam harbour area. PCBs and trace metal concentrations in both harbour sediments, and TBT, triphenyltin (TPhT) and PAH concentrations in all sediments studied, exceeded the Dutch maximum permissible concentrations (based on the protection of 95% of the ecosystem), indicating that not only are the harbours highly contaminated, but also several other locations in The Netherlands suffer from a PAH and organotin problem.

Aim of the investigation

Two transects known to exhibit contamination gradients were sampled in September 1996 [the Euromonding (Port of Rotterdam) and the North Sea Canal (Port of Amsterdam)] in order to assess the level of contamination in these harbours and to study contamination gradients. Two reference sites (Eastern Scheldt and Lake IJssel Enkhuizen) were selected as reference locations. Polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenylethers (PBDEs), nonylphenol, polyaromatic hydrocarbons (PAHs), organotin compounds and trace metals (cadmium, chromium, mercury and zinc) were determined in flounder (*Platichthys flesus*) liver and sediment from the two harbours, including the stretch along the Dutch coast between the two harbour mouths (Fig. 1). The concentrations found were compared with concentrations of the same contaminants in flounder liver and sediment from the reference locations and other areas and with background concentrations. Where relevant, reference was made to maximum permissible concentrations. This is the first time that such a comprehensive study on organic contaminants and trace metals has been carried out in both sediments and flounder liver from the two main Dutch harbours. The data will contribute to the understanding of the behaviour and fate of organic contaminants and trace metals in harbour areas in a densely populated and industrialized area in western Europe.

Materials and experimental procedures

Samples

Flounder liver. The sample locations are shown in Fig. 1. All samples were taken between the 19th and 26th September 1996. The flounders (*Platichthys flesus*) were kept alive in water from the sampling site and sacrificed within 12 h. After recording the general health condition, total fish length and weight, sex, somatic weight (gutted), liver weight and gonad weight and a brief description of the gut content, the otoliths were taken for age determination. Finally, the fishes were dissected and stored at −25 °C. The samples were divided for the various analyses in a clean air room and the livers were stored at −25 °C until
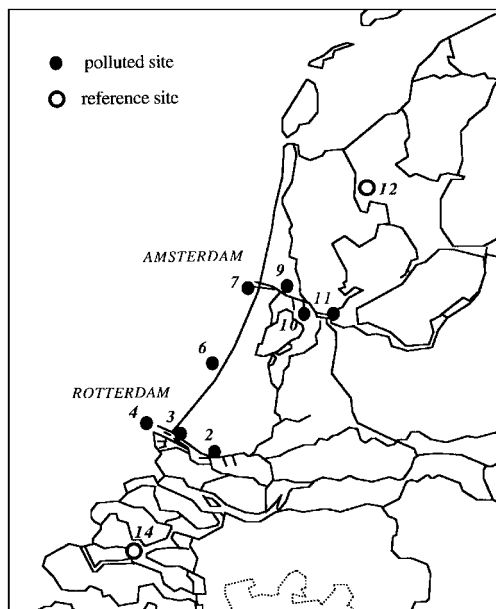
Fig. 1 Sampling locations: 2, New Waterway Benelux tunnel (Rotterdam); 3, New Waterway Splitsingsdam; 4, Loswal Noord; 6, Noordwijk; 7, IJmuiden harbour; 9, North Sea Canal Buitenhuizen; 10, Amsterdam Amerika harbour; 11, Amsterdam Oranjesluis; 12, Lake IJssel Enkhuizen (reference freshwater); 14, Eastern Scheldt (reference marine water).

analysis. Three samples were prepared for each sample location: two pooled samples of 15 flounder livers, proportionally weighed and pooled by number (two samples of 15 individual fishes of mixed sex) and one sample of 15 female flounders. The pooled samples were analysed for PCBs, OCPs and PBDEs. The female samples were analysed for nonylphenol and trace metals. Total lipid contents were determined in all samples according to Bligh and Dyer.[1] The locations Lake IJssel Enkhuizen and Eastern Scheldt served as reference locations, Lake IJssel for the freshwater environment and Eastern Scheldt for the marine environment.

Sediment. The locations of the sediment samples were identical to those of the flounder samples (Fig. 1). The sediment samples (a few centimetres in depth) were collected using a Van Veen surface sediment sampler from on board a ship. At each location, a total of 40 L of sediment was sampled. This was achieved by randomly taking five subsamples of 8 L of sediment in the area where the fish samples had been taken. Sediment samples were stored in PVC barrels for 2 weeks at 4 °C when they were homogenized for 20 min with an IKA-WERK RW-20 stirrer. Sub-samples were distributed for bioassay measurements or stored at −20 °C until chemical analysis.

Chemical analysis of the sediment sample from the North Sea Canal, Amerika harbour (location 10), showed unexpectedly low levels of contaminants, probably due to recent dredging activities in this area. The sediment from this location was therefore sampled again in September 1997. The results of the 1997 sediment analysis showed considerably higher values which were considered to be representative for this location. The results of both samples are presented.

Analytical methods

Flounder liver. PCBs, OCPs and PBDEs in flounder liver were determined according to the method of de Boer,[2,3] consisting of Soxhlet extraction with dichloromethane–pentane (1 : 1, v/v), clean-up over alumina and fractionation over silica gel; final determination was performed by gas chromatography with electron capture detection (GC-ECD) using

50 m × 0.15 mm CP Sil 19 columns. The PCB congener CB 138 was determined as CB 138 + CB 163;[4] CB 66 was determined as CB 66 + CB 95. The PBDE congeners analysed were BDE 47 (2,4,2′,4′-tetra BDE) and BDE 99 (2,4,5,2′,4′-penta BDE).

Nonylphenol was determined after exhaustive steam distillation and extraction with ethyl acetate. The sample was homogenized with a Waring blender; 10 g were taken for analysis. Water (Milli Q, 375 ml) and NaCl (24 g) were added and the sample was re-homogenized for 2 min. The sample was then subjected to six successive steam distillations, all for 1 h. After each hour, ethyl acetate was removed and collected in a centrifuge tube. After evaporation of ethyl acetate, the residue was dissolved in dichloromethane–hexane (1 : 3). The extracts were cleaned up using solid phase extraction (amino-SPE) columns (3 ml, 500 mg). After elution with 5 ml of hexane to remove interferences, the nonylphenol and ethoxylates were eluted with 5 ml of methanol. This volume was concentrated under nitrogen to 2 ml. Finally, reversed phase high performance liquid chromatography (HPLC) was carried out with fluorescence detection (excitation wavelength, 298 nm; emission wavelength, 230 nm).

The total lipid content of flounder liver was determined according to the method of Bligh and Dyer.[1]

Mercury in flounder liver was determined by flameless atomic absorption spectrometry (AAS) after microwave destruction with HNO_3 according to Pieters and Hagel.[5] Cadmium was determined using differential anodic stripping voltammetry according to Luten et al.[6] Zinc and chromium were determined with graphite oven atomic absorption spectrometry, using a PerkinElmer 5100PC spectrometer.[6]

The quality control for the various determinations in flounder liver included the use of an internal standard, analysis of an internal reference material in each sample series, incidental analysis of a certified reference material, one duplicate sample and one blank per sample series of 15 samples (trace metal analysis: all analyses in duplicate) and participation in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) proficiency testing scheme which provided four blind samples per year.[7–9] Only results for which the analytes in the internal reference material were within two standard deviations of the mean of the quality control chart were reported. Based on the long-term variance, the following relative standard deviations may be expected for the various analyses: CBs, OCPs and PBDEs, 10–25% (different per congener, dependent on concentration); nonylphenol, 25%; trace metals, 10%; total lipid, 5%.

Sediment. Sediment samples were wet-sieved over a 63 μm sieve. The fine-grained fraction was collected (Table 2, see later) using a centrifuge with a titanium rotor. Sieving results in a sample in which contaminants and standardization parameters, such as organic carbon or lutum content, are enriched and can be measured more accurately.[10] The fine-grained fractions were freeze-dried and stored dry for analysis.

PCBs in sediments were determined after Soxhlet extraction with 25% acetone in hexane, followed by alumina and silica clean-up. Cleaned extracts were analysed using high resolution GC-ECD. External calibration using an internal standard was applied to quantify the results.

PAHs were determined after Soxhlet extraction with 25% acetone in hexane, followed by alumina clean-up. Cleaned extracts were analysed using HPLC with wavelength-specific fluorescence detection. External calibration using an internal standard was applied to quantify the results.

Trace metals in sediments were analysed using a method based on a high pressure microwave digestion with nitric acid. In the resulting digest, arsenic was analysed using hydride formation and measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using external calibration.

Chromium and zinc were measured with ICP-AES using concentric nebulization. Cadmium, copper, nickel and lead were measured with ICP-AES using ultrasonic nebulization. External calibration using matrix-matched standards was applied to quantify these results. Mercury was analysed using cold vapour (CV) generation with atomic fluorescence (AF) measurement using external calibration.

Organotin compounds were determined according to Ariese et al.[11] with minor modifications. Samples (typical intake, 2 g) were thawed and acidified with excess hydrochloric acid and left overnight. After the addition of sodium chloride, the organotin compounds were extracted using diethylether with 0.3% tropolone, applying vortex mixing, sonication and phase separation in a centrifuge. The whole extraction procedure was repeated once and the two organic layers were combined. After volume reduction under nitrogen, internal standards were added and the organotin ions were derivatized in a Grignard reaction with pentyl-magnesium bromide. After volume reduction, clean-up over activated alumina and again volume reduction, the final extract was injected into the GC. Detection was via ion trap mass spectrometry with tandem MS/MS capabilities (especially useful for phenyltin compounds). External calibration was applied using ultrapure (>98%) pentylated calibrant solutions. Recovery experiments were carried out by spiking the organotin chloride salts into the wet sediment slurry (two levels in duplicate) and carrying out the complete procedure. The organotin compounds analysed were tributyltin (TBT) and its metabolites dibutyltin (DBT) and monobutyltin (MBT), as well as triphenyltin (TPhT) and its degradation products diphenyltin (DPT) and monophenyltin (MPT). Tricyclohexyltin, dicyclohexyltin and fenbutatin were also included in this study, but were not detected in any of the samples. Concentrations are expressed in nanograms of tin per gram dry weight (fine fraction) and are corrected for recovery.

Organic carbon was determined after removal of the inorganic carbon from sediment subsamples by hydrochloric acid. The treated sample sediment was burned catalytically; the evolving carbon dioxide was subsequently trapped, separated by GC and determined with thermal conductivity detection (TCD).

The quality control measurements taken for the analyses of the sediment sample fractions were similar to those taken for the flounder liver analyses. d_{12}-Perylene was used as an internal standard in the PAH analyses. Only results for which the analytes in the internal reference material were within two standard deviations of the mean of the quality control chart were reported. Based on the long-term variance, the following relative standard deviations may be expected: CBs, organotin and hexachlorobenzene (HCB), 10–25% (different per congener, dependent on the concentration); PAHs, 15–20%; trace metals, 5–10%.

The contaminant concentrations have been normalized for total organic carbon (TOC), and have been expressed on a dry weight basis, assuming they all have a 10% organic matter content (5% organic C). This was carried out to enable a proper comparison, but at the same time to keep realistic values on a dry weight basis.

Results and discussion

PCBs in flounder liver and sediment

The PCB concentrations in flounder liver on a lipid weight basis are shown in Table 1. The data are the means of two samples of 15 pooled individual flounders per location. The differences between the two samples were small and not biased to one of the pools. Only a few PCB congeners (CBs) were below the detection limits. The PCB patterns were generally comparable, but the marine samples could be distinguished from the freshwater samples, the latter showing relatively higher concentrations of lower chlorinated CBs. These lower chlorinated CBs have an increased mobility from the substrate to water and are therefore more available to aquatic organisms,[12] but, due to their volatility and their sensitivity to metabolism, disappear more easily in the marine environment with longer residence times.

The lowest PCB concentrations were found in flounder liver from the two reference samples (locations 12 and 14), but also in the samples from Noordwijk (location 6) and IJmuiden harbour (location 7), although in the flounder liver from IJmuiden harbour lower chlorinated CBs showed relatively higher concentrations than in the other samples. Loswal Noord, a former disposal site of Rotterdam harbour dredged spoils, was the marine location with the highest PCB levels. Other locations with high PCB concentrations were found in the Amsterdam and Rotterdam harbour areas, particularly at New Waterway Benelux tunnel (location 2) and North Sea Canal Buitenhuizen (location 9). There was a 4–8-fold difference in PCB concentration between the sample with the highest contamination (New Waterway Benelux tunnel) and the reference samples. Liver samples from locations 2 and 3 (Rotterdam harbour) and 9–11 (Amsterdam harbour) showed significantly higher PCB concentrations than the reference samples.

A different PCB pattern was found at the location North Sea Canal Buitenhuizen (location 9). The CB 28 concentration in flounder liver was approximately 50% of the CB 153 concentration in the same sample, whereas in other samples this ratio was approximately 10–20%. Such an elevated CB 28 concentration was not found in eels from a location near Buitenhuizen (North Sea Canal Kruithaven).[13] This difference may be explained by a higher biotransformation of CB 28 in eels, as eels can metabolize certain non-ortho-substituted CBs.[14]

CB 153 concentrations in flounder liver from the Wadden Sea (16–170 $\mu g\,kg^{-1}$ wet weight), measured for the Joint Assessment and Monitoring Programme (JAMP), were comparable with CB 153 concentrations in the marine samples in this study.[15] Flounder liver from the Western Scheldt, measured for the JAMP, showed a CB 153 concentration range of 60–430 $\mu g\,kg^{-1}$ which is closer to the CB 153 levels found in the harbour samples in the present study.[15] Marthinsen et al.[16] reported mean CB 153 concentrations of 95 $\pm$ 20 $\mu g\,kg^{-1}$ ($n = 110$), range 5.7–2088 $\mu g\,kg^{-1}$, in flounder liver from the mouth of the Glomma, south of the Oslofjord. Based on these results, the authors defined the area as slightly to moderately polluted with PCBs. PCB levels in male flounders were higher than in female flounders, 410 and 337 $\mu g\,kg^{-1}$, respectively, but the difference was not significant (one-way analysis of variance, ANOVA). They also reported that, for female flounders, significant differences were found between the sampling stations, which were not found for male flounders. Büther[17] reported total PCB concentrations in flounder liver from the German Bight and the Central North Sea: 1–5.5 $mg\,kg^{-1}$ on a lipid weight basis. The total PCB concentration in the flounder liver sample from Noordwijk in this study is estimated at approximately 1.5 $mg\,kg^{-1}$ on a lipid weight basis, which is at the lower end of the range reported by Büther.[17] Background concentrations of PCBs in flounder liver were not found in the literature. The lowest PCB concentrations in fish liver, including flounder liver, reported in the North Sea Quality Status Report were found in the northern North Sea.[18] These values, based on a total PCB (Aroclor) analysis, are around 700 $\mu g\,g^{-1}$ wet weight.

Compared to literature data, the flounder liver from the Amsterdam and Rotterdam harbours may be considered as relatively highly contaminated with PCBs, while the marine locations Noordwijk and IJmuiden have a relatively low PCB contamination.

The PCB concentrations in most sediment samples

Table 1 PCB, OCP and PBDE concentrations in pooled flounder liver ($\mu g\ kg^{-1}$ on a lipid weight basis) and total lipid contents ($g\ kg^{-1}$)

Compound	Location									
	2	3	4	6	7	9	10	11	12	14
CB 28	170	81	34	25	130	440	300	150	26	18
CB 31	99	53	24	16	76	260	190	98	17	10
CB 44	180	56	28	16	59	150	150	93	17	6.4
CB 47	150	74	27	7	52	160	120	87	10	10
CB 49	310	180	72	37	120	330	250	220	25	20
CB 52	540	280	92	46	170	450	410	330	36	30
CB 56	520	300	100	53	110	51	47	37	12	<2
CB 66 + 95	550	280	120	64	160	270	230	230	55	46
CB 87	115	72	28	18	42	96	79	110	13	6.7
CB 97	81	51	27	11	29	97	86	130	14	9.4
CB 99	600	370	150	81	140	250	210	310	72	110
CB 101	880	510	220	110	240	490	460	640	110	98
CB 105	100	67	30	20	35	100	78	79	17	29
CB 110	500	330	130	70	160	350	290	350	76	64
CB 118	710	370	180	110	160	390	310	380	85	140
CB 128	100	80	37	25	20	68	60	79	23	38
CB 137	25	16	8.1	3	<1	12	6	14	1	<1
CB 138 + 163	1300	690	350	200	280	550	450	610	180	310
CB 141	150	81	37	13	29	77	67	110	25	11
CB 149	650	420	210	95	170	360	330	480	120	160
CB 151	420	240	91	44	69	140	140	170	51	52
CB 153	1900	1000	540	270	390	830	670	950	280	500
CB 156	98	49	25	14	17	43	34	39	12	17
CB 170	300	150	85	45	40	100	78	100	55	62
CB 180	780	360	210	100	99	250	190	120	120	140
CB 187	640	320	190	100	94	210	170	200	94	150
CB 194	67	44	28	18	11	24	17	19	18	20
CB 202	88	58	30	17	20	34	29	33	8.8	17
CB 206	17	14	12	7.5	4.3	<5	<4	<5	5	4
HCB	210	77	29	36	43	190	81	59	16	12
QCB	30	12	5.6	9.7	27	1100	97	31	3.0	1.9
HCBD[a]	—[d]	—	—	—	—	<1	<1	<1	<1	<1
α-HCH	67	23	21	11	25	130	86	35	9.4	9.6
β-HCH	59	10	2.9	2.2	17	110	79	34	25	<2
γ-HCH	65	68	62	53	55	100	78	73	55	57
Dieldrin	250	140	89	77	79	93	58	30	31	64
Endrin	77	32	22	16	17	7.3	11	<4	<2	<1
p,p′-DDE	410	240	110	67	140	330	260	410	60	110
p,p′-DDD[b]	91	59	34	25	91	390	270	150	22	29
p,p′-DDT	22	7	<2	<2	7	28	<10	<7	<4	<2
o,p′-DDD	13	9.4	<1	<1	9.3	32	19	13	<4	<1
β-hepo[c]	<0.8	2.6	11	2.8	1.2	18	12	6.0	5.1	15
OCS	100	42	16	3	5.5	15	8.1	27	6.2	<1
2,4,2,4′-TBDE	280	140	60	34	41	75	57	110	36	15
2,4,5,2,4′-PeBDE	24	9	<2	<2	<3	6	<5	13	<4	<2
Total lipid/g kg⁻¹	217	139	336	275	208	254	211	184	214	203

[a]Hexachlorobutadiene. [b]Dichlorodiphenyldichloroethane. [c]Heptachloroepoxide. [d]—, not analysed.

Table 2 PCB and HCB concentrations in sediment ($\mu g\ kg^{-1}$ normalized sediment) (10% organic matter)

Compound	Location										
	2	3	4	6	7	9	10	10*[a]	11	12	14
HCB	11.2	5.0	3.0	1.3	2.9	0.9	0.5	1.7	1.7	2.8	1.0
CB 18	4.4	0.9	0.8	0.7	0.6	6.3	0.7	9.7	4.3	0.7	0.9
CB 28	12.6	7.8	3.5	3.0	7.2	10.9	0.9	12.8	9.0	6.9	1.9
CB 31	10.4	4.5	2.6	1.9	4.7	6.6	0.7	11.4	6.3	5.2	1.6
CB 44	9.0	4.7	2.8	1.4	4.1	5.8	0.6	7.3	6.7	3.5	1.0
CB 101	17.0	9.0	5.8	3.5	9.5	4.7	0.8	17.4	16.6	6.2	2.7
CB 105	2.6	2.3	1.5	1.0	2.1	0.7	0.5	2.6	1.8	1.7	1.2
CB 118	11.3	7.8	5.1	3.1	8.1	2.8	0.6	9.8	9.2	5.2	2.8
CB 138 + 163	16.3	10.9	7.7	5.1	11.6	5.0	1.6	15.1	15.5	7.4	6.1
CB 153	21.9	12.7	10.9	6.2	15.5	7.1	1.6	18.3	21.7	10.7	6.8
CB 170	7.1	4.0	2.9	1.8	4.2	1.7	0.8	4.7	8.2	3.6	2.0
CB 180	10.4	6.3	5.2	2.8	5.7	2.9	1.3	8.7	12.6	5.0	3.4
CB 187	6.9	5.8	4.4	3.0	4.7	1.8	0.5	4.6	6.8	3.0	2.4
Fine fraction (%)	14.5	66.7	3.3	1.7	4.1	21.3	44.7		48.3	26.4	55.6

[a]1997 sample.

correspond with those in flounder liver. Most PCB concentrations in the harbour sediments exceeded the Dutch maximum permissible concentrations (based on the protection of 95% of the ecosystem).[19,20] Higher PCB levels were found in sediment from the locations 2 (New Waterway Benelux tunnel) and 11 (North Sea Canal Oranjesluis) (Table 2), which corresponded to higher PCB levels in flounder liver from these locations. A discrepancy was found for location 7 (IJmuiden harbour) where PCB levels in sediment were relatively high, but this was not reflected in the flounder liver samples. Correlation coefficients for HCB and the CBs 28, 44, 101, 118, 138, 153, 170, 180 and 187 were between 0.53 and 0.84 (mean, 0.692) for all locations (with location 10* instead of 10 for sediment) for concentrations in fish on a lipid weight basis and in sediment on an organic carbon basis. However, correlation coefficients for the CBs 31 and 105 were 0.10 and −0.02, respectively. This could be caused by a different behaviour of the CBs in fish, such as the specific metabolism of these CBs.

An interesting PCB pattern was found at location 9, North Sea Canal Buitenhuizen. In both flounder liver and sediment, the lower chlorinated CBs were relatively high compared to CB 153. In the sediment sample, CB 28 showed the highest concentration of all CBs measured, whereas in most other sediment samples the CB 28 concentration was approximately 50% of the CB 153 concentration.

OCPs in flounder liver

Table 1 also summarizes the OCP concentrations in the various samples (means of two samples of 15 pooled individual flounders). The two reference samples, Enkhuizen and Eastern Scheldt, generally showed the lowest OCP concentrations. However, sometimes interesting differences were observed between the two reference locations. For example, the β-HCH concentration in the flounder liver from the Eastern Scheldt was below the detection limit, whereas 5.0–5.4 µg kg^{-1} β-HCH was found in flounder liver from Enkhuizen, due to the influence of Rhine water.[21] Rhine water also caused a higher octachlorostyrene (OCS) concentration in flounder liver from Enkhuizen, 1.0–1.6 µg kg^{-1}, compared to <0.1 µg kg^{-1} in flounder liver from the Eastern Scheldt. Dieldrin concentrations in liver from the Eastern Scheldt were approximately twofold higher than in liver from Enkhuizen. This is most probably due to the influence of the former production site of dieldrin near Rotterdam,[22] which is also reflected in the high dieldrin concentrations in flounder liver from the New Waterway Benelux tunnel (location 2) and, to some extent, from the Loswal Noord (location 4, former disposal area of dredged spoil).

The highest OCP concentrations were generally found in flounder liver from the locations New Waterway Benelux tunnel (location 2) and North Sea Canal Buitenhuizen (location 9). The North Sea Canal is known to have elevated HCH concentrations, although recently it was shown that HCH concentrations in eel and flounder liver are decreasing.[23] α-HCH and β-HCH concentrations in flounder liver from the North Sea Canal Buitenhuizen are still slightly higher than the γ-HCH concentrations. This indicates that HCH contamination, normally γ-HCH, is the highest in concentration, but the differences are all very small now and not significant, considering a relative standard deviation of approximately 20%. Büther[17] reported γ-HCH concentrations in flounder liver from the Central North Sea and the German Bight of 36–228 µg kg^{-1} on a lipid weight basis, the oldest data being from 1981. This is generally higher than that found in the present study, although the more recent data of Büther's study are closer to the present data. The γ-HCH concentration of 82 µg kg^{-1} on a lipid weight basis in flounder liver from the Belgian coast[24] is comparable to the γ-HCH concentrations found in flounder liver from the marine locations in the present study.

There were only small differences between the pooled samples per location apart from two exceptions, one of which was the HCB concentration in flounder liver from the North Sea Canal Buitenhuizen (location 9). The HCB concentration in the 16–30 pool was almost threefold higher than in the 1–15 pool. The pentachlorobenzene (QCB) concentration in flounder liver from the same location was more than sixfold higher in the 16–30 pool than in the 1–15 pool. The absolute QCB levels in flounder liver from Buitenhuizen are rather high and higher than the HCB concentrations, which may indicate local contamination. More extensive sampling is required to confirm these high QCB levels.

Büther[17] reported HCB concentrations in flounder liver fat from the German Bight and Central North Sea of 27–97 µg kg^{-1}. The range of HCB concentrations found in this study is wider, 18–280 µg kg^{-1} lipid weight, and clearly shows some HCB contaminated locations at the high end. Results from an eel monitoring programme have shown that, despite a considerable decrease in HCB concentrations in Rhine and Meuse water, HCB levels in these rivers have stabilized at a value that is still 10–100-fold higher than in other freshwater areas in The Netherlands which are not in contact with these rivers.[13] The HCB concentrations in most samples from the present study were higher than the HCB concentrations from the Wadden Sea (0.1–5 µg kg^{-1} wet weight) and the Western Scheldt (0.4–8.6 µg kg^{-1} wet weight).[15] HCB levels in some samples from the present study were comparable to those in flounder liver from the Ems-Dollard area: 4–15 µg kg^{-1} wet weight.[15]

The range of total dichlorodiphenyltrichloroethane (DDT) concentrations in flounder liver from the German Bight and Central North Sea,[17] 270–669 µg kg^{-1} lipid weight, shows an overlap with the total DDT data reported in the present study. The oldest data reported by Büther[17] are from 1991, which shows again the very persistent character of the DDT components. *p,p'*-Dichlorodiphenyldichloroethylene (*p,p'*-DDE) concentrations reported in flounder liver from the Belgian coast, 170 µg kg^{-1} on a lipid weight basis,[24] are somewhat higher than the *p,p'*-DDE values found in flounder liver from the marine locations in the present study.

HCB in sediment

HCB concentrations in sediment are summarized in Table 2. Concentrations from the locations New Waterway Benelux tunnel (location 2) and New Waterway Splitsingsdam (location 3) were approximately two- to fourfold higher than in sediment from other locations. The higher HCB concentrations in sediment correspond with the higher HCB concentrations in flounder liver from the same locations. In flounder liver, a similarly high HCB concentration was also found at location 9 (North Sea Canal Buitenhuizen), but this was not found in the sediment sample at this location.

PBDEs in flounder liver

PBDEs are used as flame retardants and some have similar environmental properties to the structurally related PCBs.[25,26] The tetra (TBDE) and penta (PeBDE) BDE concentrations in flounder liver are summarized in Table 1.

$2,4,2',4'$-TBDE was found at all locations, including the reference locations. Particularly high concentrations were found in flounder liver from the New Waterway Benelux tunnel: 280 µg kg^{-1} lipid weight. Comparison of the $2,4,2',4'$-TBDE concentrations in flounder liver from the New Waterway Benelux tunnel (location 2) with those in other flat fishes (sole liver German Bight, 2 µg kg^{-1}; plaice liver English east

Table 3 Trace metal concentrations in female flounder liver (mg kg^{-1} wet weight)

Metal	Location								
	2	3	4	6	7	9	10	12	14
Cd	0.133	0.054	0.107	0.065	0.034	0.031	0.028	0.006	0.075
Cr	<0.01	0.02	<0.01	<0.01	0.07	<0.01	—[a]	<0.01	0.02
Hg	0.18	—	0.06	0.03	0.05	0.05	0.06	0.015	0.16
Zn	50	—	66	43	40	41	—	29	51

[a] —, not analysed.

coast, 6.6 µg kg^{-1}; dab liver German Bight, 3 µg kg^{-1}),[27] shows that the TBDE concentrations found in flounder liver of the New Waterway are relatively high. The differences between the 2,4,2′,4′-TBDE concentrations in the flounder liver from the other locations are relatively small.

The two congeners which were determined here are related to the use of a so-called Penta-mix, which is primarily employed in polyurethane foams. At present, the production of this Penta-mix is decreasing.[28] A further increase in the BDEs 47 and 99 is therefore not expected. However, decabrominated diphenyl-ether, which is being used more frequently nowadays, may be expected to be present in harbour sediment samples.[25]

Nonylphenol in flounder liver

Nonylphenol concentrations in flounder liver samples were all below the limit of determination (<0.15 mg kg^{-1}). The recoveries of the nonylphenol analyses after spiking were 80–110%. Ekelund et al.[29] reported concentrations of nonylphenol in river water between 2 and 3000 µg l^{-1}. Taking into account a bioconcentration factor of 3400,[29] this would result in minimum nonylphenol concentrations in flounder liver of 6.8 mg kg^{-1} on a lipid weight basis. The results found, all <0.15 mg kg^{-1} wet weight, corresponding to <1–2 mg kg^{-1} lipid weight, indicate that the nonylphenol concentrations in water at all locations must be rather low.

Trace metals in flounder liver

Due to the very small amount of material, zinc could not be determined in two samples (location 3 and location 10). In the remaining samples, the zinc levels varied between 29 and 66 mg kg^{-1} (Table 3). Zinc concentrations in flounder liver from the North Sea Canal, determined in another study in 1997, were at a level of 28–36 mg kg^{-1}.[23] The lowest zinc concentration was found in flounder liver from the reference site Enkhuizen (location 12), but the zinc concentration in flounder liver from the other reference location, Eastern Scheldt (location 14), 51 mg kg^{-1}, was in the higher quartile of the values found. The highest zinc concentration was found in flounder liver from Loswal Noord (66 mg kg^{-1}) (location 4). The differences between the zinc concentrations in the various samples are considered to be only marginal.

The chromium concentrations were all around or just above the detection limit (Table 3). In one sample (location 10), it was not possible to determine chromium due to the low sample amount. Only in flounder liver from IJmuiden harbour was a higher chromium level found (0.07 mg kg^{-1}).

Cadmium was detected in all samples. Higher cadmium concentrations were found in flounder liver from the New Waterway Benelux tunnel (location 2) and from Loswal Noord (location 4) (Table 3). These values are in the lower range of cadmium concentrations found in flounder liver from the Western Scheldt (0.10–0.74 mg kg^{-1}).[15] The cadmium levels in flounder liver from the North Sea Canal area were lower and also lower than that in flounder liver from the reference location 6, Noordwijk (0.075 mg kg^{-1}). They were comparable to the cadmium levels in flounder liver from the North Sea Canal determined in other samples from 1996.[23] The cadmium concentration in flounder liver from the freshwater reference Lake IJssel Enkhuizen (location 12) was very low (0.006 mg kg^{-1}).

Mercury could not be determined in flounder liver from the New Waterway Benelux tunnel. The mercury content in flounder liver from location 2 was the highest of all samples (Table 3), but close to that observed in flounder liver from the reference location Eastern Scheldt (location 14). The mercury concentrations in all other samples were below 0.10 mg kg^{-1}. Mercury concentrations in flounder liver from the North Sea Canal were comparable to mercury concentrations determined in an earlier study in 1996 in North Sea Canal flounder liver.[23]

Trace metals in sediment

Somewhat elevated concentrations of cadmium and mercury were found in harbour sediments (Table 4), whereas for the other trace metals differences between harbour areas and reference locations were generally marginal. An exception was the high lead concentration at location 7, IJmuiden harbour. The somewhat higher chromium concentration which was found in flounder liver (Table 3) was not confirmed in the sediment sample of this location.

There were considerable differences in trace metal concentrations between the sediments of the two reference locations. At location 12, Lake IJssel Enkhuizen, most trace metal concentrations were higher than in sediment from location 14,

Table 4 Trace metal concentrations in sediment (mg kg^{-1} dry weight) (fine fraction, <63 µm) and total organic carbon content (%)

Metal	Location										
	2	3	4	6	7	9	10	10*[a]	11	12	14
As	16	15	14	17	29	17	11	29	41	14	13
Cd	2.34	1.46	0.88	0.81	1.92	0.90	0.24	3.02	3.16	2.90	0.38
Cr	110	93	92	100	142	76	78	109	121	75	61
Cu	47	38	21	20	77	40	18	140	129	41	10
Hg	0.69	0.53	0.34	0.30	2.43	0.54	0.08		1.24	0.65	0.16
Ni	28	27	21	20	41	37	35	41	50	25	15
Pb	72	69	57	88	272	85	34	255	234	72	30
Zn	295	231	162	238	587	269	88	648	719	422	90
% C	2.1	2.4	1.6	2.1	4.0	5.1	2.6	7.3	6.8	4.7	1.4

[a] 1997 sample.

Table 5 Organotin concentrations in sediment (µg tin kg^{-1} dry weight) (fine fraction, <63 µm)

Compound	Location										
	2	3	4	6	7	9	10	10*[a]	11	12	14
MBT	32	41	17	23	76	56	8	38	61	4.9	6
DBT	18	21	13	6.8	47	46	5.5	17	48	<2.6	1.6
TBT	38	33	13	4	52	45	6.8	15	55	3.1	1.5
MPT	15	10	7	7	53	14	<0.4	<0.8	31	<1	1.8
DPT	1.7	2.8	1	1.3	5.6	5.7	<0.4	<2.2	7.5	<0.8	1.1
TPhT	1.2	1.7	0.9	0.5	3.6	3.7	<0.3	<2.2	3.4	1	1.3

[a] 1997 sample.

Eastern Scheldt, with particularly high concentrations of cadmium (2.9 mg kg^{-1} dry weight) and zinc (422 mg kg^{-1} dry weight). Most trace metal concentrations in harbour sediments exceeded the Dutch permissible concentrations.[19,20] Both harbours are regularly dredged independent of the contaminant level and the dredged material is transferred to the North Sea. In some exceptional cases, with contaminant levels far above these maximum permissible concentrations, dredged material is deposited at special sites.

Organotin compounds in sediment

The butyltin concentrations in sediments (Table 5) of the two harbour areas were clearly higher than those in sediments from the two reference locations. However, the organotin concentrations in marine sediments from the locations Noordwijk and Loswal Noord were higher than at the reference locations. The DBT concentration was in most cases slightly lower than the MBT and TBT concentrations. The levels in the Amsterdam and Rotterdam harbour sediments were similar.

Phenyltin compounds were also detected in sediments from almost all locations, a result of the extensive use of TPhT in The Netherlands as a fungicide in potato crops. The MPT levels were usually higher than that of the parent compound, indicating degradation. All TBT and most TPhT levels in all sediments exceeded the Dutch maximum permissible concentrations.[19,20]

PAHs in sediment

Three levels of PAH concentrations in sediment could be distinguished (Table 6). Elevated PAH concentrations were found in the sediments of the entire North Sea Canal area (locations 7, 9 and 11). The PAH concentrations in sediments of the Rotterdam harbour area corresponded to the PAH concentrations in Western Scheldt sediments and to those of the reference location Lake IJssel Enkhuizen (location 12). The lowest PAH concentrations were found in sediment from the location Noordwijk (location 6), which corresponded to those of the reference location Eastern Scheldt (location 14). However, most PAH concentrations, even at the reference locations, were higher than the Dutch maximum permissible concentrations,[19,20] which shows that not only the harbours but also several other locations in The Netherlands suffer from a PAH problem.

Conclusions

The results show that the two harbours have a relatively high degree of contamination, but that specific differences between the harbours occur, both for flounder liver and sediment. In general, the patterns of the two contamination transects are similar, with lower concentrations at the marine side of the transects.

The highest concentrations of PCBs, several OCPs and PBDEs were found in flounder liver from the Rotterdam and Amsterdam harbour areas.

PCB levels in harbour sediments were generally above the Dutch maximum permissible concentrations. Compared with literature data, the PCB contamination in flounder liver from the Amsterdam and Rotterdam harbours may be considered as relatively high.

At the marine location Loswal Noord, slightly higher concentrations of higher chlorinated CBs and some OCPs were found, possibly related to its use as a harbour dredge spoil disposal site (until June 1996).

A rather high PBDE concentration was found in flounder liver from Rotterdam harbour.

The analyses of nonylphenol in flounder liver are among the first results reported on nonylphenol in biota. The nonylphenol concentrations were, however, below the detection limit, which

Table 6 PAH concentrations (µg kg^{-1} normalized sediment) (10% organic matter)

Compound[a]	Location										
	2	3	4	6	7	9	10	10*[b]	11	12	14
Ant	230	134	97	38	248	87	10	307	321	52	57
BaA	470	309	259	93	916	716	52	927	950	141	170
BaP	522	383	335	114	1157	734	58	922	950	162	189
BbF	615	470	503	163	1206	741	66	991	1014	199	256
BeP	479	358	348	121	915	553	58	827	775	163	178
BghiPe	415	337	325	152	849	506	51	649	672	177	216
BkF	243	193	199	64	497	297	28	441	379	73	94
Chr	523	305	368	101	790	695	58	946	1119	161	185
dBahA	99	71	72	30	183	122	11	139	180	30	11
Fen	513	332	576	108	601	231	43	629	1015	196	207
Flu	930	594	738	185	1551	1420	119	2156	2056	308	407
Pyr	705	494	498	137	1683	1100	90	1716	1556	218	284
InP	388	363	456	7	867	524	29	619	673	133	11

[a] Ant: anthracene; BaA: benz[a]anthracene; BaP: benz[a]pyrene; BbF: benz[b]fluoranthene; BeP: benz[e]pyrene; BghiPe: benz[ghi]perylene; BkF: benz[k]fluoranthene; Chr: chrysene; dBahA: dibenz[ah]anthracene; Fen: phenanthracene; Flu: fluoranthene; Pyr: pyrene; InP: indeno[1,2,3–cd]pyrene. [b] 1997 sample.

indicates that the nonylphenol concentrations in the water of the same locations are low.

Trace metal concentrations in harbour sediments exceeded the Dutch maximum permissible concentrations. Chromium concentrations were generally low in flounder liver. The zinc concentrations in flounder liver showed only marginal differences between the various locations. The cadmium concentrations in flounder liver showed a clear difference between the Rotterdam and Amsterdam harbour areas, the higher concentrations being found in the Rotterdam harbour area. Somewhat elevated concentrations of cadmium and mercury were found in harbour sediments, whereas for the other trace metals differences between harbour areas and reference locations were generally marginal.

The butyltin concentrations in sediments of the two harbour areas were clearly higher than those in sediments from the two reference locations. Phenyltin concentrations were considerably lower than butyltin concentrations, with the exception of MPT. TBT and TPhT concentrations in sediments from most locations exceeded the Dutch maximum permissible concentrations.

Elevated PAH concentrations were found in sediments of the entire North Sea Canal area. These concentrations were higher than those in the Rotterdam harbour area. Most PAH concentrations, even at the reference locations, were higher than the Dutch maximum permissible concentrations, which shows that not only the harbours but also several other locations in The Netherlands suffer from a PAH problem.

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References

1 E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, 1959, **37**, 911.
2 J. de Boer, *Chemosphere*, 1988, **17**, 1803.
3 J. de Boer, *Chemosphere*, 1989, **18**, 2131.
4 B. R. Larsen and J. Riego, *Int. J. Environ. Anal. Chem.*, 1990, **40**, 59.
5 H. Pieters and P. Hagel, *Biomonitoring of mercury in European eel (Anguilla anguilla) in The Netherlands, compared with pike perch (Stirostedion lucioperca): statistical analysis*, in *Trace Metals in the Environment 2: Impact of Heavy Metals on the Environment*, ed. J.-P. Vernet, Elsevier, Amsterdam, The Netherlands, 1992, pp. 203–217.
6 J. B. Luten, W. Bouquet, M. M. Burggraaf, A. B. Rauchbaar and J. Rus, *Bull. Environ. Contam. Toxicol.*, 1986, **36**, 770.
7 *QUASIMEME Laboratory Performance Studies, January–May 1995, Round 4*, SOAEFD Marine Laboratory, Aberdeen, 1995.
8 *QUASIMEME Laboratory Performance Studies, June–November 1995, Round 5*, SOAEFD Marine Laboratory, Aberdeen, 1995.
9 J. de Boer and D. E. Wells, *Mar. Pollut. Bull.*, 1996, **32**, 654.
10 F. Smedes, *Zand, slib en zeven: Standaardisering van contaminanten in mariene sedimenten, RIKZ Report 96.043*, RIKZ, The Hague, 1997.
11 F. Ariese, I. Burgers, B. van Hattum, B. van der Horst, K. Swart and G. Ubbels, *Chemische monitoring Loswal Noord-West; aanvangssituatie 1996, IVM Report 97-05*, Free University, Amsterdam, 1997.
12 S. Lacorte and M. Eggens, *Z. Angewandte Zool.*, 1992, **97**, 3.
13 J. de Boer, H. Pieters and Q. T. Dao, *Verontreinigingen in aal: monitorprogramma ten behoeve van de Nederlandse sportvsserij 1996, RIVO-DLO Rapport C048/97*, RIVO-DLO, IJmuiden, 1997.
14 J. de Boer, C. J. N. Stronck, W. A. Traag and J. van der Meer, *Chemosphere*, 1993, **27**, 1823.
15 B. L. Verboom, *Resultaten van het RWS-RIKZ NSTF/JAMP 1996 monitoring programma van bot (Platichthys flesus). Biologisch gegevens van bot en mosselen, RIVO-DLO Report C038/97*, RIKZ, The Hague, 1997.
16 I. Marthinsen, G. Staveland, J. U. Skaare, K. I. Ugland and A. Haugen, *Arch. Environ. Contam. Toxicol.*, 1991, **20**, 353.
17 H. Büther, *Arch. Fischereiwiss.*, 1990, **40**, 133.
18 *North Sea Quality Status Report 1993*, North Sea Task Force, Oslo and Paris Commissions, London, 1994.
19 *Vierde Nota Waterhuishouding*, Ministry of Transport and Public Works, The Hague, 1998.
20 N. van Straalen and A. J. Denneman, *Ecotox. Environ. Safety*, 1989, **18**, 241.
21 J. de Boer, PhD Thesis, Free University, Amsterdam, pp. 189–218.
22 M. A. T. Kerkhoff, J. de Boer, A. de Vries and P. Otte, *Negen jaren van organochloor-pesticiden onderzoek in rode aal, RIVO Rapport 86-02*, RIVO-DLO, IJmuiden, 1986.
23 J. de Boer and H. Pieters, *Analyse van microverontreinigingen in botlever en mosselen bemonsterd bij de spuisluizen te Ijmuiden, RIVO-DLO Rapport C042/97*, RIVO-DLO, Ijmuiden, 1997.
24 *North Sea Subregion 4 Assessment Report 1993*, North Sea Task Force, London, 1994.
25 A. M. C. M. Pijnenburg, J. W. Everts, J. de Boer and J. P. Boon, *Rev. Environ. Contam. Toxicol.*, 1995, **141**, 1.
26 J. de Boer, K. de Boer and J. P. Boon, in *The Handbook of Environmental Chemistry, Vol. 3, Part K, New Types of Persistent Halogenated Compounds*, ed. J. Paasivirta, Springer Verlag, Berlin, 2000.
27 J. de Boer and Q. T. Dao, *Overview of PBDE concentrations in biota and sediments, RIVO Report C020/93*, RIVO, IJmuiden, 1993.
28 J. de Boer, A. van der Horst and P. G. Wester, *Organohalogen Compd.*, 2000, **47**, 85.
29 R. Ekelund, Å. Bergman, Å. Granmo and M. Berggren, *Environ. Pollut.*, 1990, **64**, 107.