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Bioaccumulation in yellow eel (*Anguilla anguilla*) and perch (*Perca fluviatilis*) from the Dutch branches of the Rhine - mercury, organochlorine compounds and polycyclic aromatic hydrocarbons

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SUMMARY

Pollution in the Dutch branches of the River Rhine was studied through the determination of contaminant contents in yellow eel (*Anguilla anguilla*). Since perch (*Perca fluviatilis*) was very hard to catch, only a few data on this species were obtained.

Interesting differences were found between the locations in the Rhine basin, with highest contaminant levels generally found in the Haringvliet, and lowest in the Ketelmeer. Even in the Ketelmeer, levels of many contaminants were, however, strongly elevated compared to the Lauwersmeer.

Of the compounds studied -mercury, organochlorine compounds and polycyclic aromatic hydrocarbons-, mercury, PCBs and HCB cause most concern. For the former two compounds, Dutch consumption standards are, in some cases, exceeded, while HCB contents exceed the West-German consumption standard by a factor of 2. "Ecotoxicological values" [1] are exceeded for PCBs, HCB and Σ p,p'-DDT by factors of 10, 7 and 2, respectively.

The pollution situation in the Haringvliet is of particular concern. Relatively high levels of contaminants have been found is this area for many years. However, in 1988 a severe deterioration was observed, in particular for mercury, PCBs and DDT related compounds.

1. INTRODUCTION

For more than 10 years, the Netherlands Institute for Fishery Investigations (RIVO) has determined contents of, mainly organic, contaminants in yellow eel (*Anguilla anguilla*) from different waters in the Netherlands. The primary reason for this research is concern for the human consumption of eel. However, eel is also very suitable as an indicator organism for the levels of organic contaminants in the aquatic environment. The main reasons are the omnipresence of this species, its high lipid content, which leads to pronounced bioaccumulation of lipophilic compounds, and the fact that it is, until early summer, rather stationary.

The establishment of a Rhine Action Plan has given a renewed impulse to the use of eel as an indicator organism. As part of this Plan, the Institute for Inland Water Management and Waste Water Treatment (DBW/RIZA), the National Institute for Public Health and Environmental Protection (RIVM) and RIVO have set up a coordinated research program on the "Ecological Rehabilitation of the River Rhine". This program is build on three main themes : bio-alarm systems, basic exploration, and impact assessment [2]. The study described in this report forms part of the latter theme.

Through the establishment of the research program "Ecological Rehabilitation of the River Rhine", an extension of the contaminant studies by RIVO has become possible. Both the number of locations and the number of compounds studied were enlarged. This study was made possible through the collaboration of RIVO and DBW/RIZA, under DBW/RIZA contract no. DB-456. The RIVM contributed to this study by performing analyses of eel samples for polychlorodibenzo-p-dioxins and polychlorodibenzofurans.

The contaminant levels in eel contribute to the information on various types of impact of pollution. In the first place, it gives an indication of the burdening of fishes by pollutants, which could be of importance to sensitive species like the salmon [3]. In the second place, eel is a part of the natural food chain in the riverine ecosystem. Contaminated eel can form a heavy burden to fish-eating birds like cormorants [4] and aquatic mammals like the otter [5]. Finally, of course, the contaminant contents are important to the human fish consumer. Although the Rhine Action Plan does not specifically mention this, a goal of the Plan should be that the fish present in the river is fit for consumption.

Since the accumulation of mercury is not possitively correlated with lipid content and because in some piscivorous feeders, like perch (*Perca fluviatilis*) and pikeperch (*Stizostedion lucioperca*), mercury accumulates to higher extents than in eel, the accumulation of this element in perch was studied, too. Because mercury contents in fish are highly related to length and weight [6], eel were analysed in three different length-classes.

2. MATERIALS AND METHODS

2.1. Sampling

Fishing for yellow eel (*Anguilla anguilla*) and perch (*Perca fluviatilis*) was performed, in May 1988, at the locations indicated in figure 1. In this way information should be obtained on all Dutch branches of the Rhine. The Lauwersmeer was included as a relatively clean reference area. Eel was caught using electric fishing equipment, perch using a bottom trawl. Unfortunately, it proved impossible to catch enough eel in the Nieuwe Waterweg at Maassluis. Perch proved to be very hard to catch. Numbers of fish caught, length and weight data are given in table 1. Samples were stored at - 20 °C until analysis.
For the organic analyses, pooled samples of equal weights of eel of approximately 30 - 40 cm length were used. For the mercury analyses, eel samples were divided in three subsamples with length-classes of < 30 cm, 30 - 40 cm and > 40 cm.

2.2. Analyses

All analyses, except for chlorophenols and polycyclic aromatic hydrocarbons (PAHs), were performed in duplicate, *i.e.* the complete analytical procedures were applied to subsamples of one pooled sample. The results of duplicates were generally within a 5 % range for the mercury analyses and within 10 % for the organic analyses, except for the PCDD and PCDF determinations.

2.2.1. Mercury

Aliquots of 0.9 g for perch and 0.4 g for eel were used for digestion. The digestion of the fish tissues was performed at high pressure and temperature (150 ^{o}C) for four hours using "Uniseal" destruction vessels in the presence of 6 ml nitric acid (65 %, Suprapur, Merck). After cooling of the destruction vessels nitrogen oxides were expelled with N_2 gas and the clear solution was made up to 10 ml with bidistilled water. During the reduction of the mercuric ions with tin (II) chloride (10 % g/v) no mixing of the solution was performed. The released mercury was analysed by cold flameless atomic absorption spectrometry using a LDC/Milton Roy Mercury Monitor. The peak from the mercury vapour, measured at 253.7 nm, was used to determine the mercury concentration in fish.

2.2.2. Organochlorine compounds, except PCDDs, PCDFs and chlorophenols

Samples were homogenized in a Waring blender. Circa 5 g homogenate was ground with Na_2SO_4 to dryness and subsequently soxhlet extracted during 6 hrs. with 100 ml dichloromethane/n-pentane (1/1 v/v). After evaporation of dichloromethane and adjustment to 100 ml with n-pentane, the lipid content was determined by evaporating 10 ml extract to dryness and weighing the residu. For eel, the lipid content determined in this way is approximately equal to that determined according to Bligh & Dyer [7].

An amount of extract containing 250 mg lipid was cleaned up over a 15 g Al_2O_3.6 % H_2O column, eluting with n-pentane, to remove the lipids. After addition of 2 ml iso-octane and reducing the volume to 2 ml by evaporation, the extract was transferred to a 1.8 g SiO_2.1½% H_2O column and fractionated in two portions eluting with 11.5 ml iso-octane and 10 ml 15 % diethylether in iso-octane (v/v), respectively. The first fraction contained PCBs, HCBD, QCB, HCB, p,p'-DDE, heptachlor and chlordenes, the second one p,p'-DDD, p,p'-DDT, HCHs, dieldrin, endrin, hepo, chlordanes, oxychlordane, t-nonachlor, α-endosulfan and chloronitrobenzenes.

After dilution or concentration and addition of octachloronaphtalene (OCN) (0.04 mg/ml) as an internal standard, 0.5 ml of each fraction was injected by an autosampler in the gas-chromatograph. Results were corrected for recovery, which was 75 - 105 %.

The following GC conditions were used :

GC	: Perkin-Elmer 8500
column	: 50 m x 0.15 mm i.d. CP Sil 8 CB (Chrompack), film thickness 0.20 µm
carrier gas	: H_2 at 0.3 ml/min
injection	: split/splitless at 270 °C
detector	: ^{63}Ni, 370 MBq, ECD at 360 °C

oven program I (all compounds except CNBs)

90 °C, 2 min
30 °C/min → 215 °C
215 °C, 40 min
5 °C/min → 270 °C
270 °C, 23 min

oven program II (chloronitrobenzenes)

90 °C, 2 min
3 °C/min → 170 °C
5 °C/min → 270 °C
270 °C, 40 min

2.2.3. Polychlorodibenzo-p-dioxins and -dibenzofurans

These analyses were performed by RIVM. The method is briefly described here; the method is based on the procedure as described by Liem *et al.* [8]. PCDDs and PCDFs were isolated from 25 g gram of an eel homogenate by soxhlet extraction during 20 hours with toluene. Prior to extraction, a mixture of $^{13}C_{12}$ labeled PCDD and PCDF reference standards were added to the sample material in the extraction thimbles. The extract was cleaned up by using consecutively an activated carbon column, a multilayer column (silica, silica-H_2SO_4, silica, silica-NaOH, silica, silica-$AgNO_3$) and an alumina column. In general, the same clean-up procedure was used as reported by Liem *et al.* [8], except that n-hexane was used instead of petroleum ether. Prior to the GC/MS analysis, 4,4'-dibromobiphenyl was added as an internal standard. Concentrations were determined by gaschromatography / mass spectrometry on a VG 70SQ tandem hybrid mass spectrometer coupled to a Hewlett Packard 5890A gaschromatograph. A CP-Sil 88 fused silica capillary column (50 m x 0.22 mm i.d., film thickness 0.12 μm) was used. The mass spectrometer was operating under electron impact conditions at a resolving power of 4000 in the Selected Ion Monitoring mode. Results were corrected for recoveries, which were 35 - 80 %. Detection limits were found to be approximately 0.1 ng/kg fillet.

2.2.4. Chlorophenols

Preconcentration of the chlorophenols has been performed by a distillation-extraction (DE) method. The DE apparatus was composed of a single distillation set-up and a seperation chamber with a drain-cock. The refluxing water was flowing through the extraction solvent (hexane), so that a continuous extraction process was established. A side-arm connecting the seperation chamber with the distillation set-up allowed backflow of condensed water into the sample reservoir. The hexane layer (2 ml) was refreshed three times within a total process time of 3 hours.

After the distillation-extraction process the isolated chlorophenols have to be converted into their acetate derivatives prior to GC/MS analysis [9]. For that purpose the hexane was extracted two times with 1.5 ml 0.1 N NaOH. The pooled NaOH extracts were transferred into a test-tube, containing 3 ml borax buffer (4 %, pH = 9.2), 2 ml 0.1 μg/ml tetrachloronaphtalene (TCN) (internal standard) in iso-octane and 100 μl acetic anhydride. The test-tube was immediately shaken for 1 min. After centrifugation the iso-octane solution, containing the derivatized chlorophenols, was dried with a few crystals of sodium sulphate.

A Hewlett-Packard (HP 5993) gaschromatograph/mass-spectrometer was used for the analytical work, applying a capillary quartz column CP-Sil 8CB of 25 m length for the separation. The following conditions were used :

column	: 25 m x 0.24 mm i.d. CP-Sil 8CB film thickness 0.44 µm
injection	: split/splitless at 260 °C, 1 µl by hand
carrier gas	: helium, flow 0.6 ml/min (u = 24 cm/s)
septum purge	: helium, 0.5 ml/min
temperatures	: transferline : 280 °C source : 180 °C analyser : 220 °C
oven program	: 90 °C, 1.5 min 10 °C/min → 230 °C 230 °C, 12.5 min
detector mode	: Selected Ion Monitoring (SIM)
ionisation	: electron impact 70 eV

For the acetates of the chlorophenols the following masses were used for quantification and identity check, respectively:

phenol	: 94.0, 136.0
monochlorophenols	: 128.0, 130.0
dichlorophenols	: 162.0, 164.0
trichlorophenols	: 196.0, 198.0
tetrachlorophenols	: 232.0, 230.0
pentachlorophenol	: 265.9, 263.9
tetrachloronaphtalene (internal standard)	: 265.9, 263.9

To evaluate the recovery rates, samples were cleaned up, derivatized and analysed by GC/MS with and without the addition of 50 µg/kg chlorophenols (spike). The results are listed in table 2. Recovery rates showed a standard deviation of 12 - 17 %. Variations due to the preparation of the sample, the DE process and the GC/MS analysis are included in these figures.

The detection limit, calculated as a peak greater than 3 fold the base noise, was estimated as 2 µg/kg for pentachlorophenol (1 µg/kg) and 1 µg/kg for 2,4-dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol (0.5 µg/kg), using 10 (20) g fish material.

2.2.5. Polycyclic aromatic hydrocarbons (PAHs)

These analyses were performed by the TNO-Institute for Fishery Products. Eel homogenates (30 g) were shaked with ethanolic KOH for 3 hrs. at 37 °C. PAHs were extracted with hexane and the extract was cleaned up over an alumina/silica column. After evaporation to dryness and dissolution in acetonitrile, the PAHs were determined by reversed phase HPLC, using a fluorescence detector. The excitation wavelength was 350 nm and emission was measured at 410 nm, except in case of indeno[1,2,3-c,d]pyrene, for which 380 and 500 nm, respectively, were used.

3. RESULTS AND DISCUSSION

3.1. Mercury

The mercury contents of nine eel samples, collected in 1988 from the Dutch part of the River Rhine basin are given in table 3. In figure 2 the mercury contents are depicted in a histogram.

Low levels of mercury (< 0.25 mg/kg Hg) were observed for the Rhine at Lobith, Waal, IJssel, Ketelmeer and Lauwersmeer. Eel from the Lauwersmeer exhibited the lowest mercury contents. Medium levels were measured for the Lek at Krimpen and Nieuwe Merwede. In eel from the Haringvliet mercury levels were strongly elevated. Except for the Haringvliet, mercury levels in eel remain clearly below the consumption standard of 1 mg/kg product.

Subsamples eel of length > 40 cm showed highly increased mercury contents in comparison to the subsamples eel of smaller length, except for the Rhine and the Waal locations. At these locations only minor differences in mercury content between the subsamples were found. The lack of difference in mercury level between the two smaller length classes of eel, which has been observed for all sample sites, is noteworthy. For pikeperch the increase of mercury content with length in young fish is more pronounced [6].

From the data in table 3 it is evident that mercury contents of eel in the western part of the Rhine basin are at a higher level in comparison to the Rhine at Lobith. In 1986 and 1987 at sites more downstream from the Rhine at Lobith mercury contents of eel have been observed to be at an elevated level, too [10]. Moreover, in pikeperch from the Hollands Diep a trend of steady increasing mercury concentrations during the last five years could be estimated [11]. In this area no industrial activity is known to exist. The continuing sedimentation of heavily contaminated suspended matter can act as a main source of mercury pollution in this area. However, active release of mercury from new sources cannot be excluded.

Eel from the Haringvliet, collected at a location east of the isle of Tiengemeten exhibited very high mercury contents. In section 3.2.4. further comments on this area are made.

Mercury data for perch from several locations in the Dutch Rhine basin are given in table 4. The mean lengths of the samples differ considerably. For that reason comparison of the mercury contents of perch from different sites is hardly possible. However, the level in eel from Ketelmeer appears to be more elevated than from the Rhine at Lobith.

In 1981 the mercury contents in perch from the Haringvliet and Hollands Diep have been measured at a level of 0.65 mg/kg wet weight [12]. In comparison to the findings in this

report (table 4), the mercury level in perch has been reduced in the period from 1981 to 1988.
The difference in mercury contents between eel and perch from the Haringvliet is remarkable. It is not clear whether this is caused by small differences in fishing locations or by the different life-style of these fishes.

3.2. Organochlorine compounds

The results of these determinations are given in table 5.

3.2.1. Polychlorobiphenyls (PCBs)

The contents of 24 polychlorobiphenyl (PCB) congeners in eel were determined. High PCB contents were found, showing a maximum level of nearly 9 mg/kg wet weight in eel from the Haringvliet for the sum of 24 components (Σ_{24}). The measured contents in fish from the River Rhine are *circa* 20 times those in the Lauwersmeer, where Σ_{24} still is 200 µg/kg wet weight. The Dutch human health standards [13] are exceeded at several locations (figure 3). It has to be borne in mind that these standards are derived from an Acceptable Daily Intake of 1 µg PCBs per kg body weight per day, which has been considered as too high [14]. The "ecotoxicological values" of Stortelder *et al.* [1] (see table 6), based on the risk to specialized fish predators like the otter, are exceeded by a factor of 10.
Regarding possible effects, the levels of the mono-ortho-substituted CB-105 (2,3,3',4,4'-pentachlorobiphenyl) and CB-118 (2,3',4,4',5-pentachlorobiphenyl) are of interest. Besides the highly toxic non-ortho-substituted PCBs, this type is considered relatively dangerous, too [15, 16]. The levels of non-ortho-substituted PCBs are below the detection limits of the employed method.
Between the locations, some differences in contents are observed. Contents of selected congeners are shown in figure 4. Firstly, the high levels in eel from the Haringvliet are striking. Although the levels were rather high in preceeding years, too, this year a drastic increase has occurred. This increase has been confirmed in a renewed study in the autumn of 1988. In the section on DDT related compounds (3.2.4.) further comment is given on this issue.
The interesting phenomenom occurs that levels in the IJssel branch (at Deventer and Ketelmeer) are relatively low compared to the Rhine and Waal branches. This was not observed in freshwater mussels (*Dreissena polymorpha*) [17]. An explanation is not easily given. The exact fishing location and the local food supply for the eel probably exhibit an influence.

Except from the mentioned increase in the Haringvliet, PCB levels show only minor changes over the years (figure 5). At Lobith, less chlorinated biphenyls are slightly decreasing, in contrast to the constant higher chlorinated congeners. In the Hollands Diep hardly any decrease is seen. In the Ketelmeer, levels were remarkably low in 1988. It has to be awaited whether this trend will continue. For the Haringvliet, the different trend for different congeners is noteworthy. The highly chlorinated congeners have drastically increased.

The observed contents are presented in an alternative fashion in figure 6, on lipid basis and as percentage of Σ_{24}. Contents in eel from the IJsselmeer [18] and from the Haringvliet (1987) [19] are included for comparison. It appears that in the ratios between the various congeners remarkably little variation occurs, despite the different samping locations. The patterns in eel from the Haringvliet (1988) and the Lauwersmeer are different from the others. They suggest exposure to a more weathered PCB mixture, in which less chlorinated biphenyls have diminished. This weathered PCB pattern also shows a relatively low CB-149 and a relatively high CB-153 content.

Despite the attention which PCBs have got for years, contents of these compounds are invariably high. Clear indications of ecotoxicological effects, in particular on fish-eating mammals in both freshwater (otter [5]) and marine ecosystems (seal [20, 21]), have been noted and consumption standards are exceeded. Furthermore, large amounts of PCBs are still in use or dumped on land, which could eventually enter the aquatic environment [15]. Reduction of the severe load of these compounds to the Rhine should be considered of priority importance. Concentrations should decrease at least 10 times.

3.2.2. Hexachlorobenzene (HCB), pentachlorobenzene (QCB) and hexachlorobutadiene (HCBD)

HCB, QCB and HCBD form part of the waste from industrial chemical processes in which organic compounds are chlorinated. Although the contents of these compounds in eel have drastically decreased during the early 1980's, this decrease has stopped in recent years (figure 7). The measured contents are, even now, markedly higher (i.e. 30 x) than those in eel from a clean area as the Lauwersmeer (figure 8).

Apparently, no further reduction of the discharges of these compounds is effectuated. However, HCB levels should still be considered much too high. Contents in eel from the River Rhine are a factor of 2 above the consumption standard in the Federal Republic of Germany[1] of 500 µg/kg lipid [22]. The "ecotoxicological value" [1], which leads to 140 µg/kg lipid (table 6) is exceeded 7 times.

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[1] These are mentioned for comparison where no Dutch standards are available.

An explanation for the high HCBD content found in the Ketelmeer is not known.

3.2.3. Hexachlorocyclohexanes (HCHs)

The observed contents of HCHs are shown in figure 9 on a lipid base. For these compounds the difference between the contents in the River Rhine basin and in the Lauwersmeer are less pronounced. Contents in freshwater mussels (*Dreissena polymorpha*) showed a comparable situation [17]. The observed data suggest an elevated input of lindane (γ-HCH) to the Hollands Diep. A clear trend in time is not observed for the HCH contents in eel (figure 10).
HCHs appear to be ubiquitous contaminants. Since bioaccumulation factors are much smaller than those of PCBs or HCB [23], rather high HCH concentrations could well be present in the aquatic environment of the eel. The observed γ-HCH contents in eel are, however, clearly below the West-German standard of 2000 µg/kg lipid [22].

3.2.4. DDT and related compounds

The observed p,p'-DDT, p,p'-DDE and p,p'-DDD contents are shown, on a lipid base, in figure 11. The very high p,p'-DDE and p,p'-DDD contents in eel from the Haringvliet are the most striking feature. Those figures are much higher than in previous years (figure 12), and were confirmed in a renewed determination in the autumn of 1988. The combined strong increase of these contents and of the mercury and PCB contents suggest that either substantial amounts of complex chemical waste have recently been dumped in this area or severe turbation and resuspension of contaminated sediment upstream (Nieuwe Merwede, Hollands Diep) has occurred , e.g. through dredging.
The high p,p'-DDT contents at Krimpen aan de Lek attract attention, too. Further research to clarify the origin of this high level is recommendable.
Unfortunately, the DDT related compounds are, $2\frac{1}{2}$ decades after "Silent Spring" [24], still present in relatively high contents. Except for the Haringvliet, no clear time trend is observed (figure 12). In other European waters comparable contents have been found, e.g. 900 - 1900 µg/kg lipid for p,p'-DDE in brown trout (*Salmo trutta*) from Lake Geneva (1984) [25], and 400 - 1400 µg/kg lipid for p,p-DDE in pike (*Esox lucius*) from the Finnish Lake Päijänne (1980) [26]. In the North American Great Lakes much higher contents were determined (1985) in lake trout (*Salvelinus namaycush*), ranging from 90 µg/kg <u>wet</u> weight in Lake Superior to 900 µg/kg <u>wet</u> weight in Lake Ontario for Σ DDT [27].

The contents found in this study are well below the West-German consumption standard of 3500 µg/kg wet weight for the sum of the three compounds mentioned [22]. However, Σ p,p'-DDT exceed the "ecotoxicolgical value" [1], which comes down to 400 µg/kg lipid (table 6), by a factor of 2.

3.2.5. Endrin and dieldrin

Endrin was not detected in any of the samples. Dieldrin, however, was clearly present (figure 13). Contents are 5 - 10 times below the West- German standard (1000 µg/kg lipid [22]) and, on lipid basis, 2 - 5 times below the contents in lake trout from the remote Siskiwit Lake on an isle in Lake Superior [28]. The influence of the River Rhine appears to be limited for this compound. No trend in time is observed (figure 14). The somewhat elevated levels at Krimpen aan de Lek and in the Hollands Diep/Haringvliet area should be noted.

3.2.6. α-Endosulfan

α-Endosulfan was not detected in any sample, in contrast to the determinations in freshwater mussels [17]. Metabolism of this compound is probably the cause.

3.2.7. Chloronitrobenzenes

Although it is known that chloronitrobenzenes can be metabolized by fish [29], these compounds have been detected in fish from the River Main [30] and from the Mississippi [31]. Since these compound are present in the Rhine [32], it was tried to determine these in the sampled eels. None of the studied isomers was, however, found above the detection limit of *circa* 1 - 10 µg/kg wet weight. Perhaps more information on the presence of these compounds in the environment could be obtained from determinations in *Dreissena*.

3.2.8. Heptachlor, chlordane and related compounds

From table 5h it is apparent that the contents of these compounds in eel are very low. Only heptachlorepoxide and cis-chlordane were found at several locations, while in the Haringvliet trans-chlordane and trans-nonachlor were detected, too. These pesticides are, as far as known, not used in Western Europe, in contrast to Japan and America. In Japan much higher contents in fish (*circa* 10 mg/kg lipid for total chlordanes) have been found at various locations [33].

3.2.9. Polychlorodibenzo-p-dioxins and -dibenzofurans (PCDDs and PCDFs)

The results of the measurements by RIVM are given in table 5i. The observed levels are very low indeed. They correspond well with the measurements by Van den Berg *et al.* [4]. Haringvliet is somewhat elevated, Lauwersmeer somewhat lower compared to the other locations. The relatively less toxic octachlorodibenzo-p-dioxin is the major component in all samples. However, in toxicity equivalents 2,3,7,8-TeCDD, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD are the most important congeners (figure 15). The observed levels are 2.5 - 8 times lower than the Canadian consumption standard of 20 ng/kg [34].
PCDDs and PCDFs are subjected to (slow) metabolism in fish [35]. Considering the toxicity of these compounds, it would therefore be interesting to know the levels in eel livers, too. These could be of importance to the intake of PCDDs and PCDFs by fish predators.

3.2.10. Chlorophenols

The concentrations of four chlorophenols in eel are included in table 5j. The figures are corrected for the recovery rate of an added spike and calculated as µg/kg on productbasis, rounded off to micrograms. In all eel tissues low amounts of chlorophenols have been determined at a concentration level below 10 µg/kg.
2,4 - Dichlorophenol and 2,4,6 - trichlorophenol could be detected at a level two to four fold the detection limit. Pentachlorophenol could only be detected as traces, except for eel from the Haringvliet. In Ketelmeer and Lauwersmeer minor traces of 2,4 - dichloro- and pentachlorophenol have been determined (indicated as 'trace' in table 5j).
For phenol and pentachlorophenol low recovery rates for the distillation-extraction process were found. These results are in agreement with the findings of Rijks *et al.* (1983), who obtained recoveries between 17 and 70 % for phenol and methyl-substituted phenols [36]. The low recovery rates for distillation-extraction of phenols are inherent in the polar properties of this class of compounds and their strong interaction with water.
The contents of chlorophenols in eel from Dutch waters collected in 1988, varied between 2 and 7 µg/kg fresh weight. For pentachlorophenol only trace amounts have been detected. Measurements of PCP in eel, collected in 1983, showed values between 13 and 48 µg/kg, the content of eel from the River Rhine at Lobith being 18 µg/kg [19]. Evidently, since 1983 contents of chlorophenols in eel have drastically decreased , reflecting the decrease of these compounds in the river Rhine water, which in 1987 had diminished to about 20 % of the 1983 levels [37].
Eel from the Haringvliet exhibited the highest levels of chlorophenols in 1988. Data for chlorophenols in the sediments of several surface waters in the western part of the river

Rhine basin, obtained from a sampling project in 1977 [38], also showed the highest contents for chlorophenols to be present in the sediments of the Haringvliet area.
Chlorophenols have been widely used as herbicides, fungicides and wood preservatives. The last decade the levels of chlorophenols in water and organisms from the river Rhine basin have considerably decreased to concentrations near the detection limit, due to banning measures performed by many countries.
In comparison to fish filet, liver tissue of perch show contents of chlorophenols which are 5 - 10 times higher. Therefore, the use of fish liver as target organ is suggested for future monitoring of chlorophenols in the environment.
It can be concluded that, despite a considerable decrease of the levels in the last decade and their moderate affection for lipids, chlorophenols are still found in freshwater fish from several sites in the River Rhine ecosystem.

3.3. Polycyclic aromatic hydrocarbons (PAHs)

Since it is known that fish are well able to metabolize these compounds [39,40], it is not surprising that hardly any PAH was detected in the eel samples (table 7).

4. CONCLUSIONS

Experience over the years has shown that yellow eel can be very well used for monitoring time trends of lipophilic compounds. It appears to be suited for monitoring mercury, too. This species has the advantage of being abundantly available, in contrast to perch, which could hardly be caught in the branches of the River Rhine.

Apart from giving information on time trends, contents in eel also reflect to what extent contaminants are really taken up from the environment. In this respect, interesting differences were found between the various locations, which are, however, difficult to explain.

Using eel as an indicator species, the following results were obtained. In 1988 a severe deterioration of the contaminant situation (Hg, PCBs, ΣDDT) has occurred in the Haringvliet. Over the whole Dutch Rhine basin, levels of polychlorobiphenyls (PCBs) and hexachlorobenzene (HCB) should be considered as too high. Despite its ban, DDT related compounds are still present in relatively high concentrations, indicating their extreme persistence. Polychlorodibenzo-p-dioxins (PCDDs) and - dibenzofurans (PCDFs) were found in very low amounts, below human consumption standards. However, these compounds could be of toxicological importance to fish predators.

Concentrations of endrin and chlordane related compounds are very low. The same holds for α-endosulfan, chloronitrobenzenes and chlorophenols, but possible metabolism of these compounds has to be taken in account. Polycyclic aromatic hydrocarbons (PAHs) were almost absent from all eel samples. Due to metabolism, this species is not a useful indicator for these compounds.

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Table 1a : Sample data on yellow eel

Sample location	< 30 cm			30 - 40 cm			> 40 cm		
	Average length (min - max) (cm)	Average weight (min - max) (g)	N	Average length (min - max) (cm)	Average weight (min - max) (g)	N	Average length (min - max) (cm)	Average weight (min - max) (g)	N
Rijn, Lobith	25.5 (21 - 31)	34 (19 - 62)	25	35.4 (31 - 41)	100 (75 - 136)	25	44.8 (35 - 50)	214 (103 - 296)	25
Waal, Tiel	26.2 (23 - 29)	36 (19 - 54)	25	32.8 (29 - 37)	81 (60 - 120)	25	43.3 (35 - 53)	176 (121 - 266)	3
Lek, Krimpen	29.3 (24 - 32)	51 (35 - 65)	24	35.2 (30 - 39)	98 (66 - 132)	25	49	264	1
Nieuwe Merwede	26.6 (23 - 30)	35 (24 - 56)	25	33.3 (31 - 37)	79 (60 - 103)	25	50 (41 - 64)	230 (112 - 410)	3
Hollands Diep	27.1 (24 - 29)	43 (30 - 57)	25	32.7 (31 - 38)	74 (50 - 96)	25	60 (46 - 72)	508 (192 - 859)	8
Haringvliet	28.7 (27 - 30)	45 (34 - 58)	25	32.8 (30 - 37)	73 (59 - 109)	25	51 (48 - 56)	277 (235 - 326)	3
IJssel, Deventer	28.0 (24 - 30)	39 (24 - 51)	25	35.2 (32 - 43)	92 (65 - 150)	25	--	--	
Ketelmeer	27.8 (24 - 31)	40 (29 - 60)	25	35.0 (31 - 39)	102 (66 - 135)	24	--	--	
Lauwersmeer	26.0 (24 - 29)	34 (26 - 38)	25	29.8 (27 - 35)	54 (42 - 75)	25	--	--	

Table 1b : Sample data on perch

Location	Average length (min - max) (cm)	Average weight (min - max) (g)	N
Rijn, Lobith	19.3 (15 - 26)	123 (42 - 599)	13
Nieuwe Merwede	15.0 (12 - 16)	55 (25 - 82)	3
Hollands Diep	27.8 (23 - 33)	326 (169 - 618)	8
Haringvliet	28.0 (24 - 37)	400 (203 - 1019)	20
IJssel, Deventer	16.1 (14 - 20)	66 (37 - 116)	6
Ketelmeer	23.0 (18 - 33)	190 (84 - 375)	26

Table 2 : Recovery rates for chlorophenols

Compound	Recovery rate (%)	RSD (%)
2,4 dichlorophenol	100	16
2,4,5 trichlorophenol	73	17
2,4,6 trichlorophenol	90	17
pentachlorophenol	26	12

number of measurements : 9

Table 3 : Mercury contents in yellow eel (mg/kg fillet)

Location	Length class		
	< 30 cm	30 - 40 cm	> 40 cm
Rijn, Lobith	0.20	0.25	0.15
Waal, Tiel	0.23	0.22	0.19
Lek, Krimpen	0.34	0.27	0.66
Nieuwe Merwede	0.42	0.34	0.78
Hollands Diep	0.23	0.29	nd
Haringvliet	0.92	0.96	1.36
IJssel, Deventer	0.22	0.24	0.66
Ketelmeer	0.18	0.24	nd
Lauwersmeer	0.16	0.16	nd

nd : not determined

Table 4 : Mercury contents in perch

Location	mg/kg fillet
Rijn, Lobith	0.22
Nieuwe Merwede	0.24
Hollands Diep	0.42
Haringvliet	0.41
IJssel, Deventer	0.26
Ketelmeer	0.38

Table 5a : PCB contents in yellow eel (µg/kg fillet)

PCBno.:		28	31	44	47	49	52	66 + 95	87
location	lipid (g/kg)								
Rijn, Lobith	176	16	6.4	64	90	51	190	140	42
Waal, Tiel	243	13	5.6	77	75	54	190	170	66
Lek, Krimpen	235	18	4.8	86	130	53	180	150	54
Nieuwe Merwede	194	12	4.1	70	120	53	150	140	50
Hollands Diep	196	13	8.3	35	180	40	130	140	47
Haringvliet	163	<20	12.0	76	180	52	160	180	62
IJssel, Deventer	176	7.2	3.1	38	39	16	83	65	21
Ketelmeer	279	9.7	3.3	34	35	22	100	69	27
Lauwersmeer	190	1.7	0.5	1.7	2.5	0.81	4.7	5.4	1.5

PCBno.:	97	101	105	110	118	128	138	141	149
location									
Rijn, Lobith	44	270	54	230	200	53	420	58	430
Waal, Tiel	54	340	76	290	210	61	560	88	670
Lek, Krimpen	51	330	91	270	310	72	510	68	410
Nieuwe Merwede	55	360	91	270	320	81	690	87	480
Hollands Diep	41	290	110	260	330	75	610	70	340
Haringvliet	59	400	180	360	650	180	1500	120	550
IJssel, Deventer	20	130	40	110	100	29	250	34	240
Ketelmeer	20	120	36	120	110	28	200	31	150
Lauwersmeer	0.7	11	4.2	8.6	13	4.5	30	4.5	13

Table 5a (continued) : PCB contents in yellow eel (µg/kg fillet)

PCBno.:	151	153	170	180	187	194	206
location							
Rijn, Lobith	65	570	110	210	300	19	3.6
Waal, Tiel	110	620	130	220	480	22	3.9
Lek, Krimpen	62	760	130	200	220	22	5.3
Nieuwe Merwede	67	920	140	290	300	34	8.5
Hollands Diep	42	910	160	270	250	36	6.2
Haringvliet	78	2200	340	640	610	70	11
IJssel, Deventer	35	320	51	100	190	12	2.5
Ketelmeer	25	260	42	79	93	8.8	2.7
Lauwersmeer	2.6	49	12	16	13	2.2	0.82

Table 5b : HCB, QCB and HCBD contents in yellow eel (µg/kg fillet)

location	lipid (g/kg)	HCB	QCB	HCBD
Rijn, Lobith	176	210	78	46
Waal, Tiel	243	300	130	81
Lek, Krimpen	235	280	120	50
Nieuwe Merwede	194	200	79	40
Hollands Diep	196	160	49	16
Haringvliet	163	130	nd	15
IJssel, Deventer	176	140	59	36
Ketelmeer	279	120	25	73
Lauwersmeer	190	5.8	2.2	0.32

Table 5c : HCH contents in yellow eel (µg/kg fillet)

location	lipid (g/kg)	α-HCH	β-HCH	γ-HCH
Rijn, Lobith	176	10	16	46
Waal, Tiel	243	17	23	74
Lek, Krimpen	235	9.9	12	59
Nieuwe Merwede	194	8.3	13	48
Hollands Diep	196	12	16	100
Haringvliet	163	9.1	nd	70
IJssel, Deventer	176	6.8	9.1	38
Ketelmeer	279	20	nd	73
Lauwersmeer	190	5.4	6.5	23

Table 5d : Contents of DDT and related compounds in yellow eel (µg/kg fillet)

location	lipid (g/kg)	p,p'-DDE	p,p'-DDD	p,p'-DDT
Rijn, Lobith	176	130	49	9
Waal, Tiel	243	110	52	20
Lek, Krimpen	235	180	85	100
Nieuwe Merwede	194	140	57	14
Hollands Diep	196	140	62	21
Haringvliet	163	550	110	13
IJssel, Deventer	176	73	28	12
Ketelmeer	279	120	55	nd
Lauwersmeer	190	31	22	4.6

nd : not determined

Table 5e : Endrin and dieldrin contents in yellow eel (µg/kg fillet)

location	lipid (g/kg)	endrin	dieldrin
Rijn, Lobith	176	<9	16
Waal, Tiel	243	<14	23
Lek, Krimpen	235	<8	51
Nieuwe Merwede	194	<3	23
Hollands Diep	196	<6	33
Haringvliet	163	<4	35
IJssel, Deventer	176	<4	15
Ketelmeer	279	<2	20
Lauwersmeer	190	<3	11

Table 5f : α-Endosulfan contents in yellow eel (µg/kg fillet)

location	lipid (g/kg)	α-endosulfan
Rijn, Lobith	176	<5
Waal, Tiel	243	<8
Lek, Krimpen	235	<3
Nieuwe Merwede	194	<3
Hollands Diep	196	<7
Haringvliet	163	<5
IJssel, Deventer	176	<3
Ketelmeer	279	<8
Lauwersmeer	190	<1

Table 5g : Contents of chloronitrobenzenes in yellow eel (µg/kg fillet)

location	lipid (g/kg)	1-Cl-3-nitro	1-Cl-4-nitro	1-Cl-2-nitro	1,4-diCl-2-nitro	1,3-diCl-4-nitro	1,2-diCl-4-nitro	1,2-diCl-3-nitro	pentaClnitro
Rijn, Lobith	176	<2	<5	<3	<4	<1.5	<2	<0.9	<0.6
Waal, Tiel	243	<3	<4	<5	<4	<3	<3	<2	<0.9
Lek, Krimpen	235	<3	<4	<2	<1.5	<2	<2	<1	<1
Nieuwe Merwede	194	<2	<3	<2	<1	<1.4	<2	<0.8	<0.9
Hollands Diep	196	<2	<7	<2	<2	<2	<3	<1.4	<0.9
Haringvliet	163	<5	<6	<4	<6	<4	<2	<1	<0.7
IJssel, Deventer	176	<3	<4	<2	<2	<2	<2	<1	<1
Ketelmeer	279	<4	<10	<6	<5	<3	<3	<2	<1
Lauwersmeer	190	<3	<2	<1	<0.7	<0.8	<1	<0.5	<0.5

Table 5h : Contents of heptachlor, chlordane and related compounds in yellow eel (µg/kg fillet)

location	lipid (g/kg)	heptachlor	heptachlorepoxide	cis-chlordene	trans-chlordene	oxychlordane	cis-chlordane	trans-chlordane	trans-nonachlor
Rijn, Lobith	176	<7	nd	<5	nd	<3	5.3	<1	<3
Waal, Tiel	243	<9	nd	nd	nd	<2	nd	<2	<5
Lek, Krimpen	235	<0.6	3.9	<0.7	nd	<2	1.8	<1	1.8
Nieuwe Merwede	194	<3	3.6	<4	nd	<1	<1	<1.4	<1
Hollands Diep	196	<10	nd	<8	nd	<9	7.7	<2	<4
Haringvliet	163	<20	5.9	<5	nd	<7	5.3	2.9	6.1
IJssel, Deventer	176	<3	2.6	<4	nd	<2	<1.5	<2	<1.5
Ketelmeer	279	<4	3.2	nd	nd	<2	8.3	<2	<5
Lauwersmeer	190	<0.6	2.7	<0.7	nd	<1	<0.7	<0.7	nd

nd : not determined

Table 5i : Contents of polychlorodibenzo-p-dioxins and polychlorodibenzofurans in yellow eel (ng/kg fillet)

		Rijn, Lobith	Nieuwe Merwede	Hollands Diep	Haringvliet	IJssel, Deventer	Lauwersmeer
	lipid (g/kg) :	176	194	196	163	176	190
isomer	toxicity-factor						
2,3,7,8-TeCDD	1	1.8	1.5	2.6	3.4	1.8	0.8
2,3,7,8-TeCDF	0.1	1.8	5.3	5	4.5	3.8	1.6
1,2,3,7,8-PeCDF	0.05	0.1	0.2	0.2	0.2	0.2	0.1
2,3,4,7,8-PeCDF	0.5	1	1.5	1.8	2.1	1.6	0.8
1,2,3,7,8-PeCDD	0.5	1.1	1.4	1.1	2	1.1	0.9
1,2,3,4,7,8-HxCDF	0.1	0.8	1.5	1.5	2.2	1.4	0.6
1,2,3,6,7,8-HxCDF	0.1	0.4	0.6	0.6	0.9	0.6	0.3
1,2,3,7,8,9-HxCDF	0.1	0.4	0.8	0.6	1.1	0.7	0.4
2,3,4,6,7,8-HxCDF	0.1	0.1	<0.1	<0.1	<0.1	0.1	0.1
1,2,3,4,7,8-HxCDD	0.1	0.3	0.4	0.2	0.2	0.3	0.4
1,2,3,6,7,8-HxCDD	0.1	2.5	5.3	4	7.5	3.9	3.4
1,2,3,7,8,9-HxCDD	0.1	0.6	3	0.7	3.5	1.4	0.7
1,2,3,4,6,7,8-HpCDF	0.01	2	3.2	2.7	3.9	2.4	1.1
1,2,3,4,7,8,9-HpCDF	0.01	0.3	0.5	0.3	0.8	0.4	0.1
1,2,3,4,6,7,8-HpCDD	0.01	3.3	6.6	3	9.4	5	4.1
OCDF	0.001	0.6	0.3	0.3	0.3	0.2	0.1
OCDD	0.001	15	12	16	21	12	13
2,3,7,8-TCDD-equivalents		3.62	4.77	5.4	7.61	4.47	2.47

Table 5j : Contents of chlorophenols in yellow eel (µg/kg fillet)

location	amount analyzed (g)	number of determinations	lipid (g/kg)	2,4-dichloro-phenol	2,4,5-trichloro-phenol	2,4,6-trichloro-phenol	pentachloro-phenol
Rijn, Lobith	20	2	176	2	n.d.	2	trace
Waal, Tiel	10	2	243	3	n.d.	2	trace
Lek, Krimpen	10	1	235	3	n.d.	2	trace
Nieuwe Merwede	20	1	194	3	n.d.	3	trace
Hollands Diep	10	1	196	4	n.d.	2	4
Haringvliet	10	3	163	4	2	4	7
IJssel, Deventer	10	1	176	3	n.d.	2	trace
Ketelmeer	10	1	279	trace	n.d.	n.d.	n.d.
Lauwersmeer	10	1	190	trace	n.d.	trace	trace

n.d. : not detected

Table 6 : Ecotoxicological values [1][1]

Compound	Ecotox. value for water (ng/l)[2]	log K_{ow} [1]	Ecotox. value for fish (µg/kg lipid)[3]
PCB-28	0.48	5.62	200
PCB-52	0.064	6.1	80
PCB-101	0.12	6.11	160
PCB-118	0.014	7.07	160
PCB-138	0.080	6.4	200
PCB-153	0.054	6.57	200
PCB-180	0.046	6.72	240
HCB	0.093	6.18	140
Σ p,p'-DDT	0.25	"6.2"	400

Remarks:

1. "Ecotoxicological values" are designed to protect living communities in the aquatic environment. These values correspond with laboratory based no-observed effect levels for populations of aquatic organisms or their predators.
2. Relates to the dissolved fraction.
3. Calculated according to $C_{lipid} = C_{water} \times K_{ow}$.

Table 7 : Contents of polynuclear aromatic hydrocarbons (Borneff series) in yellow eel (µg/kg fillet)

location	lipid (g/kg)	fluoranthene	benzo[b]-fluoranthene	benzo[k]-fluoranthene	benzo[a]pyrene	benzo[g,h,i]-perylene	indeno[1,2,3-c,d]-pyrene
Rijn, Lobith	176	4	<1	<1	<1	<1	<1
Waal, Tiel	243	3	<1	<1	<1	<1	<1
Lek, Krimpen	235	2	<1	<1	<1	2	<1
Nieuwe Merwede	194	2	<1	<1	<1	<1	<1
Hollands Diep	196	4	<1	<1	<1	<1	<1
Haringvliet	163	3	<1	<1	<1	<1	<1
IJssel, Deventer	176	3	<1	<1	<1	<1	<1
Ketelmeer	279	4	<1	<1	<1	<1	<1
Lauwersmeer	190	3	<1	<1	<1	<1	<1

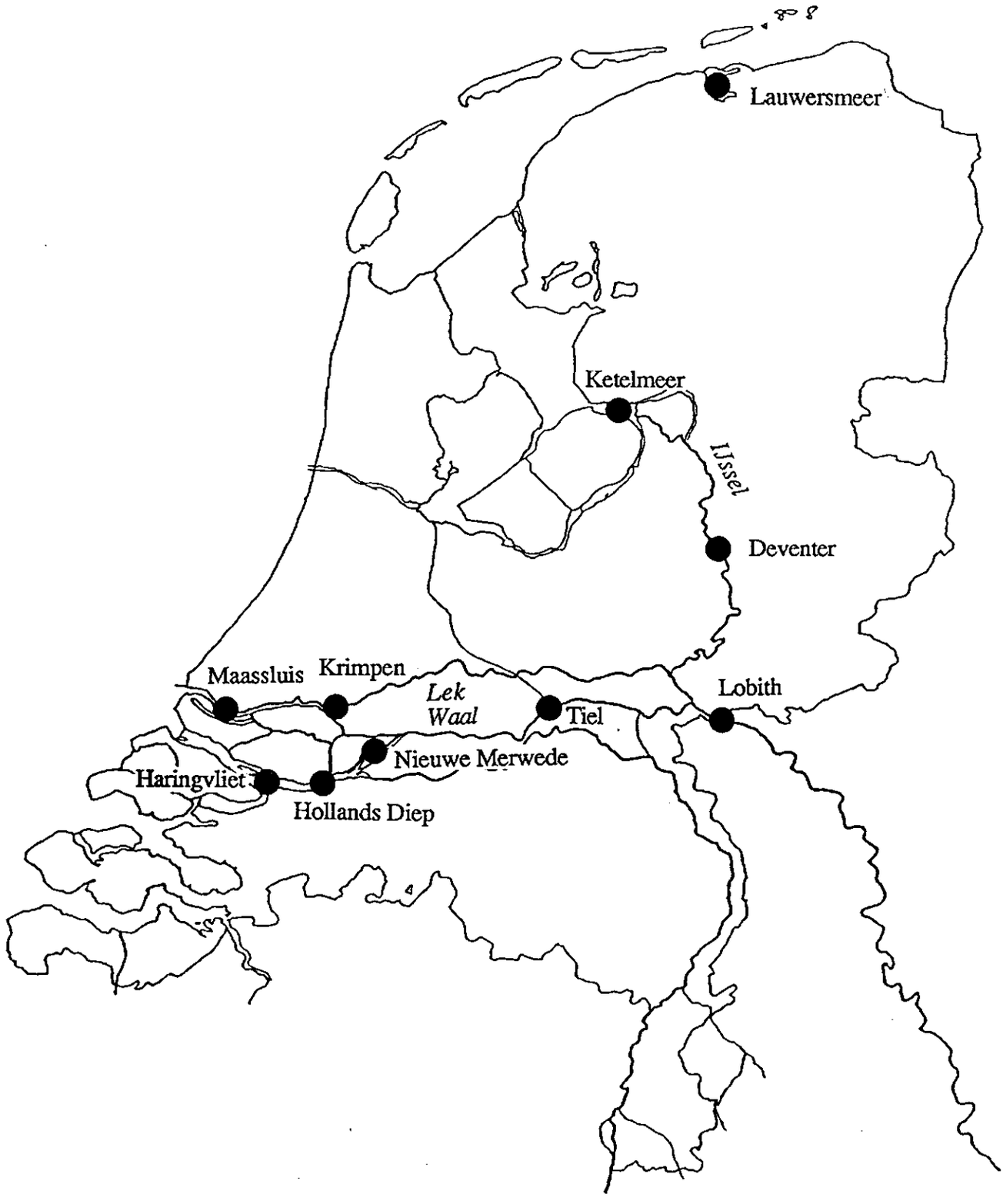

Figure 1 : Sampling locations

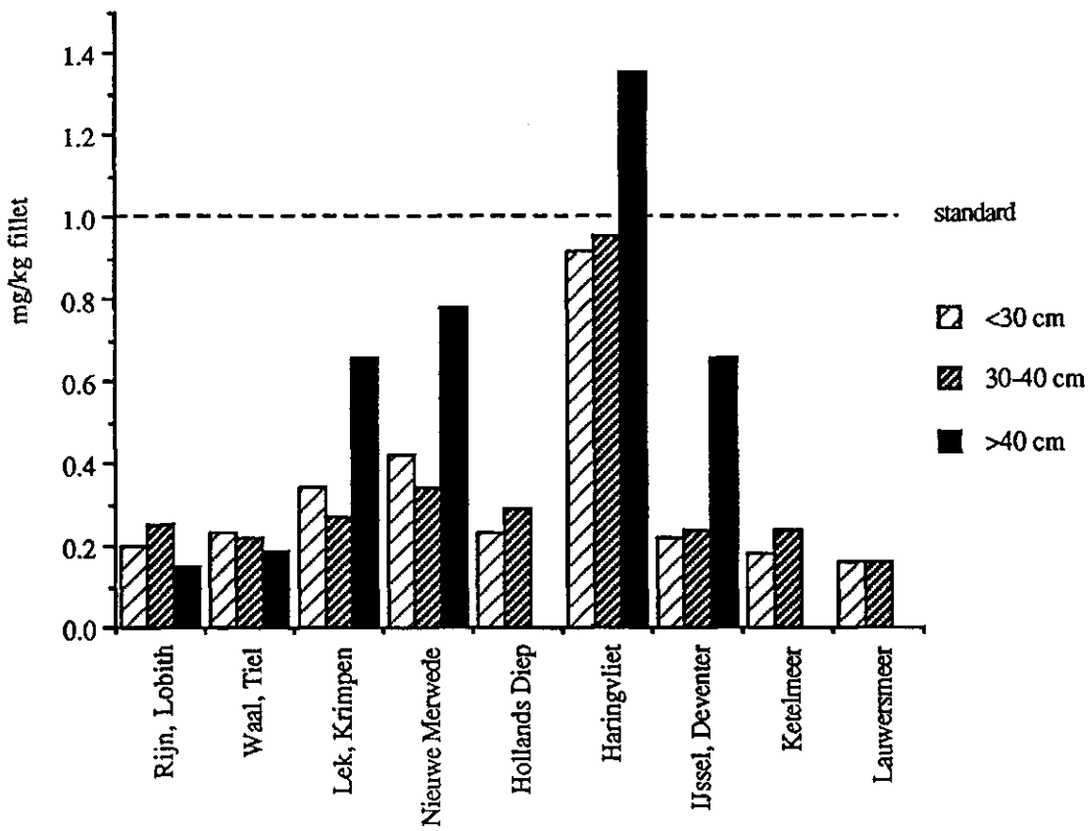

Figure 2 : Mercury contents in yellow eel, on wet weight basis

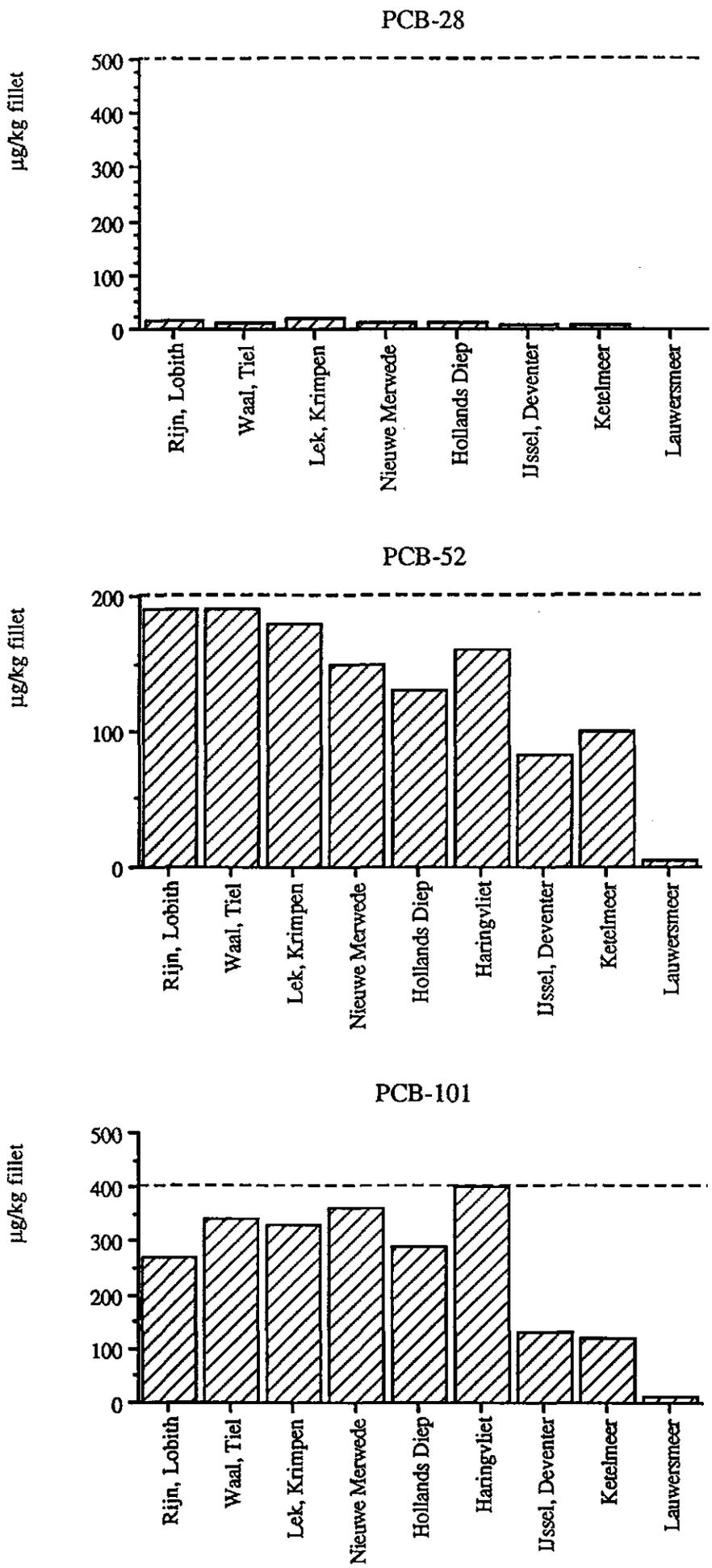

Figure 3 : PCB contents in yellow eel compared to the consumption standard

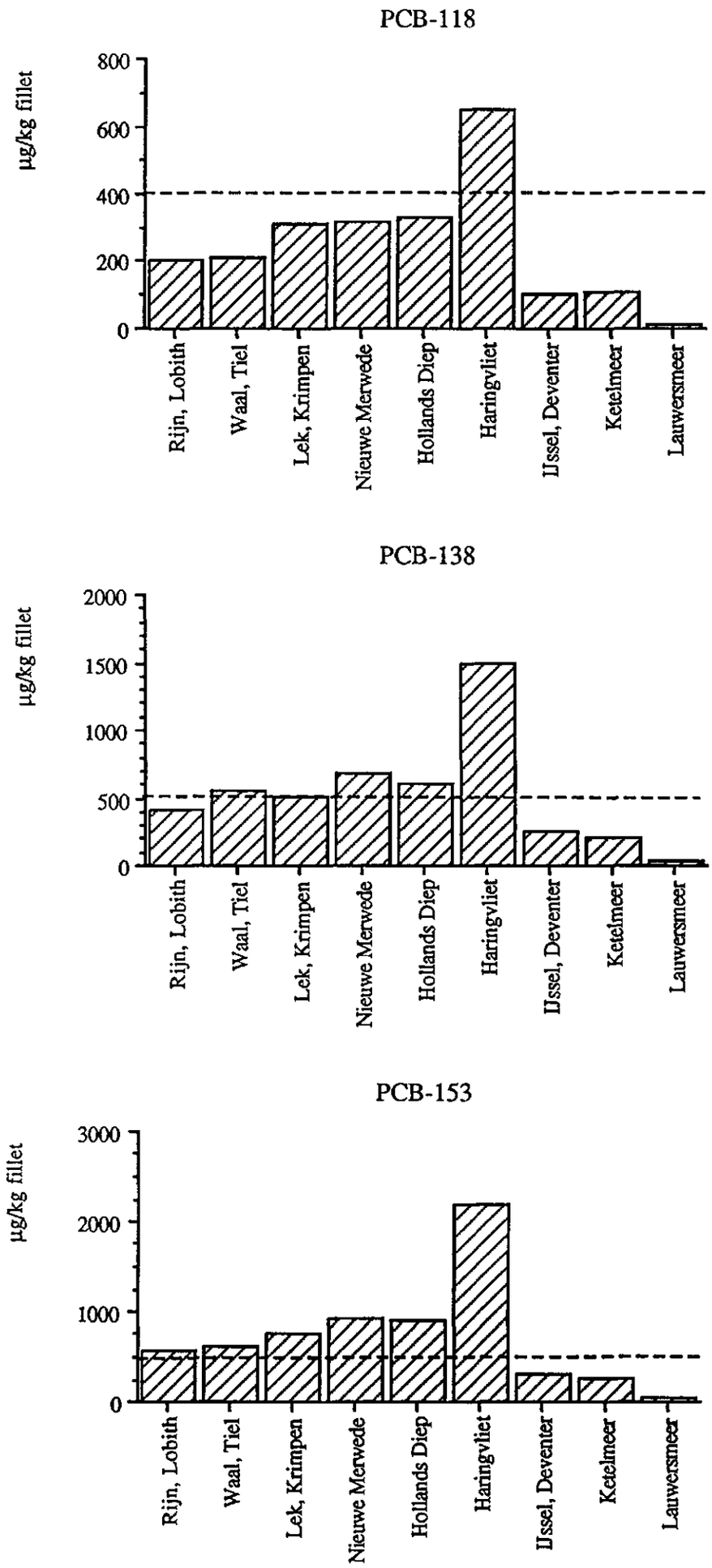

Figure 3 (continued) : PCB contents in yellow eel compared to the consumption standard

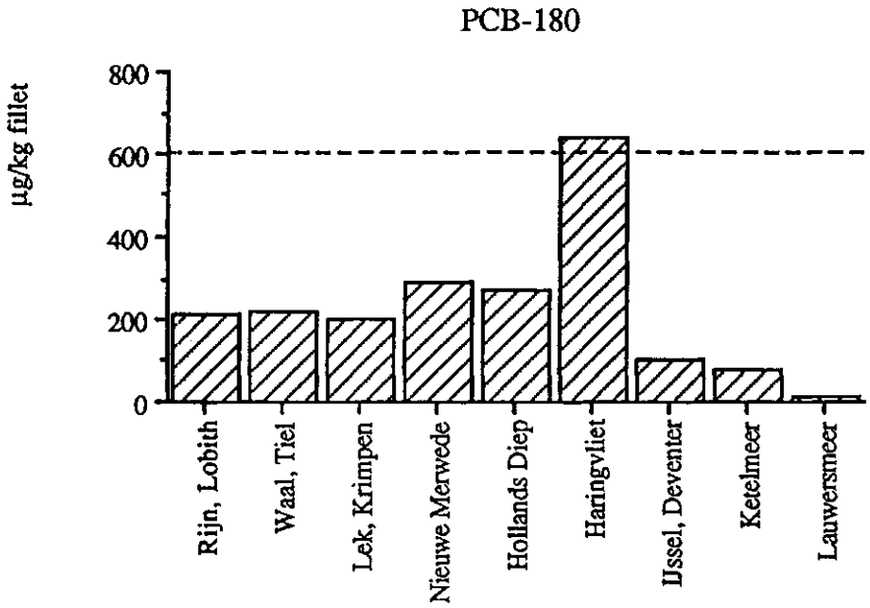

Figure 3 (continued) : PCB contents in yellow eel compared to the consumption standard

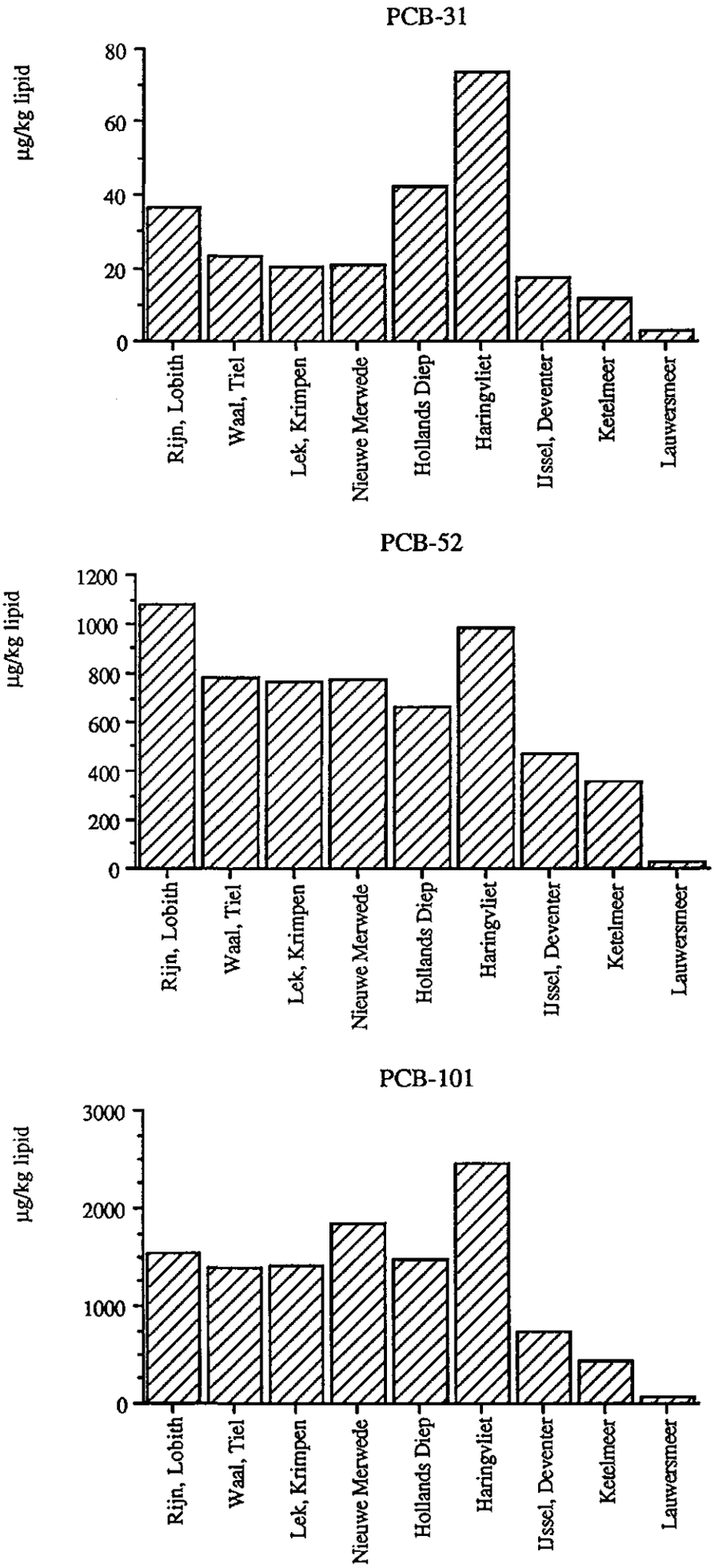

Figure 4 : PCB contents, on lipid basis, at different locations

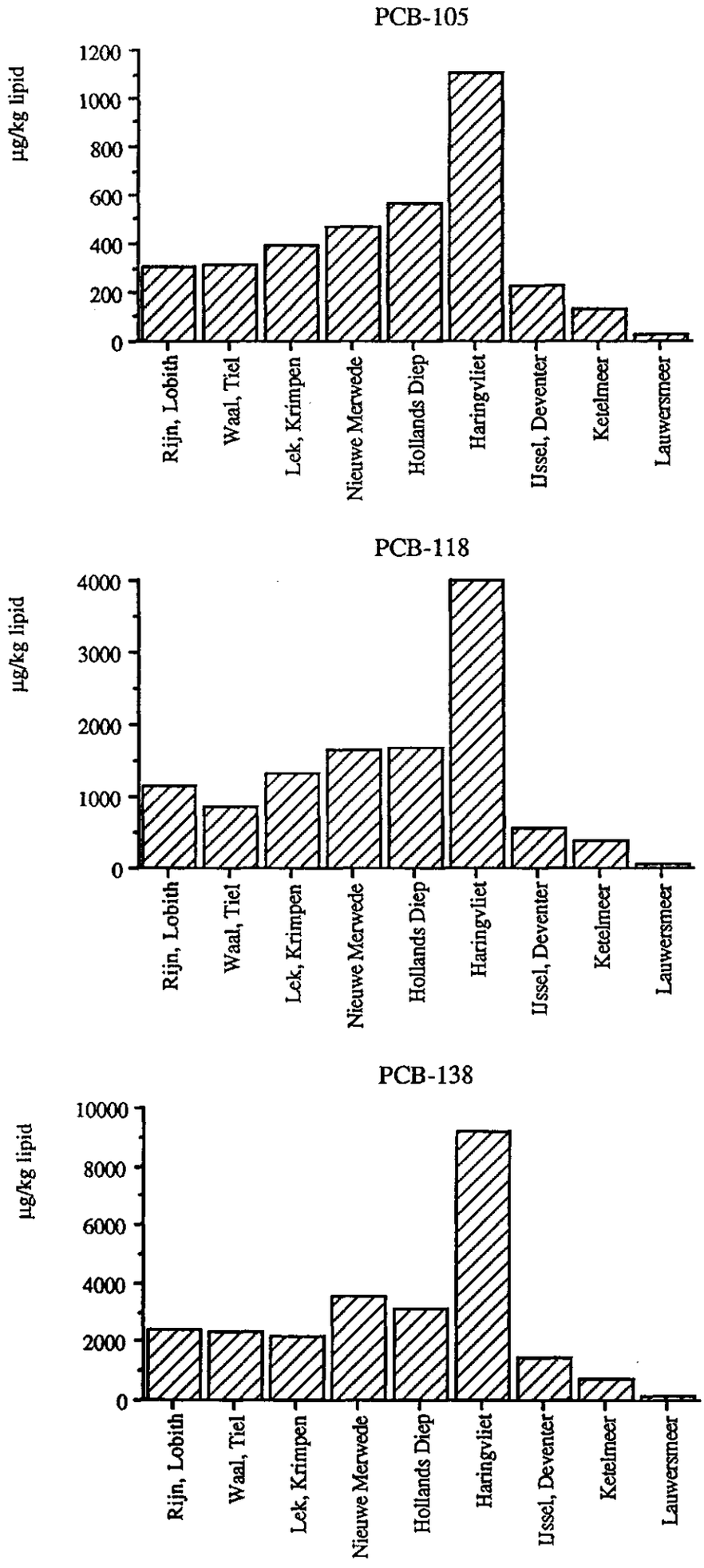

Figure 4 (continued) : PCB contents, on lipid basis, at different locations

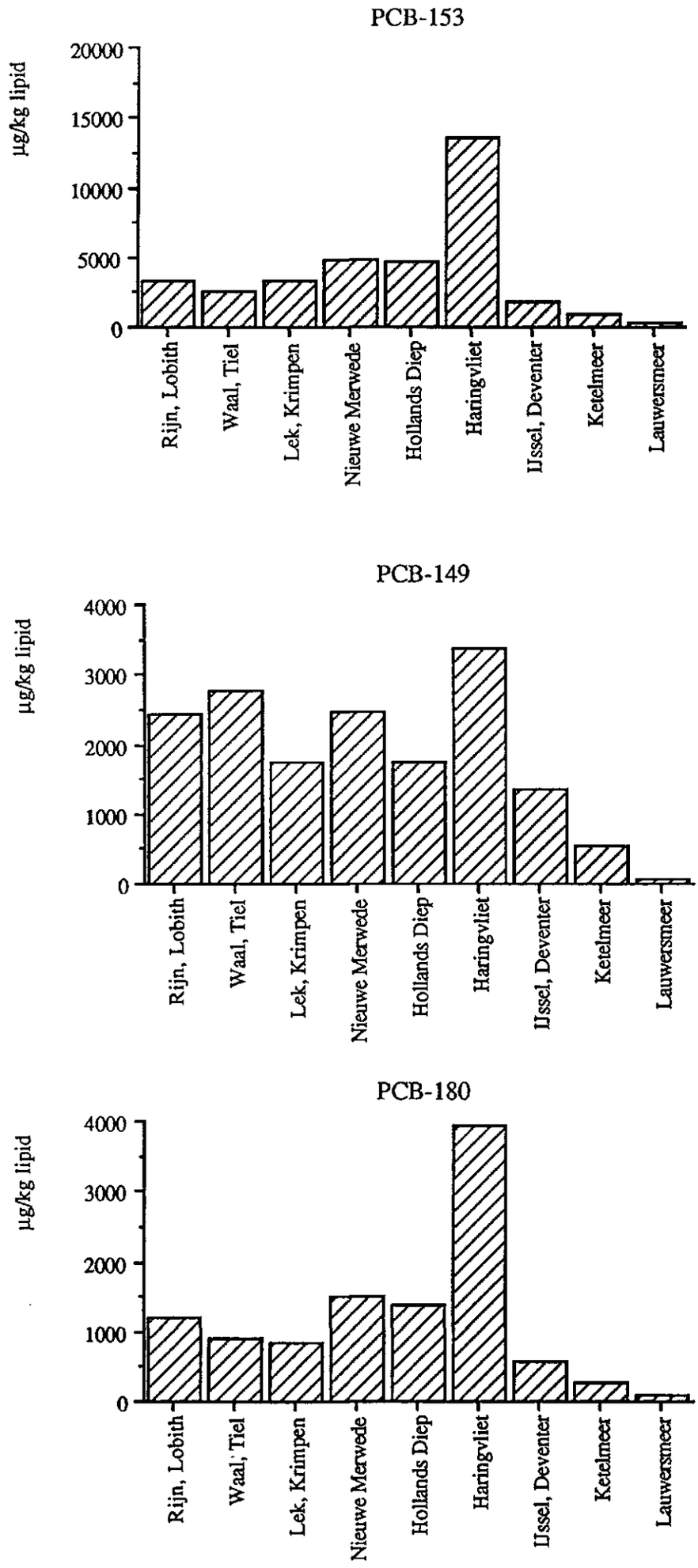

Figure 4 (continued) : PCB contents, on lipid basis, at different locations

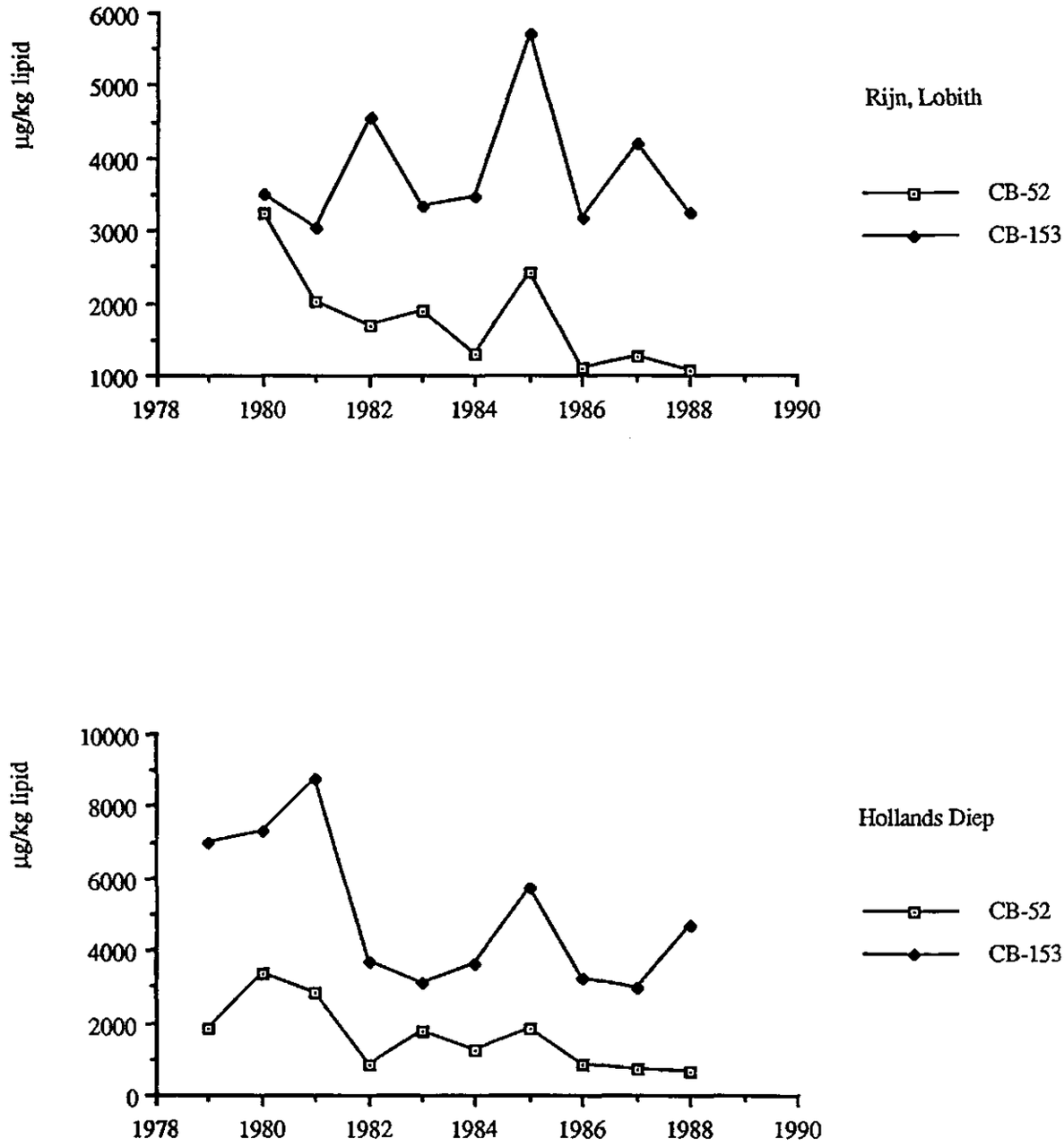

Figure 5 : Selected PCB time trends in yellow eel

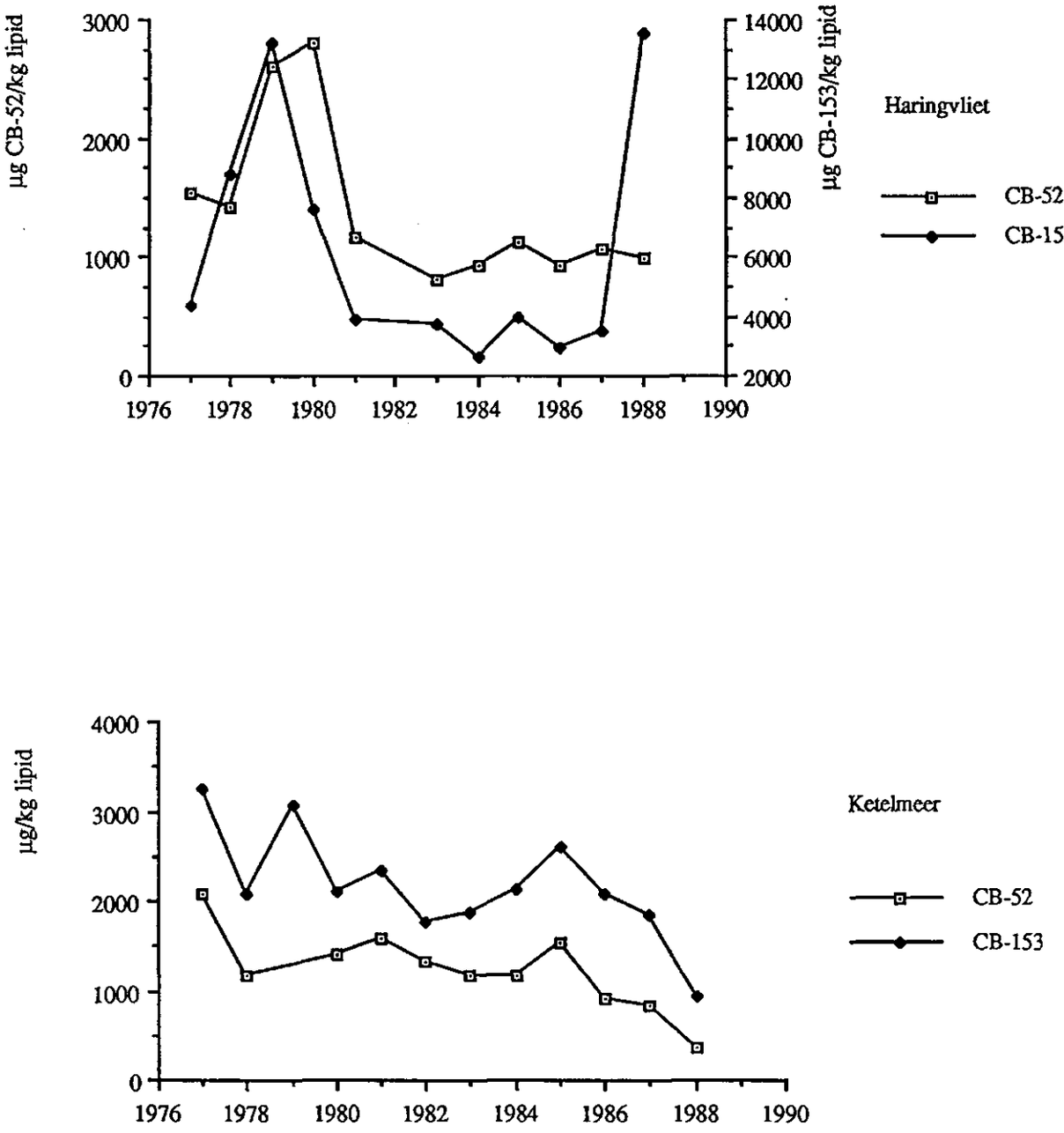

Figure 5 (continued) : Selected PCB time trends in yellow eel

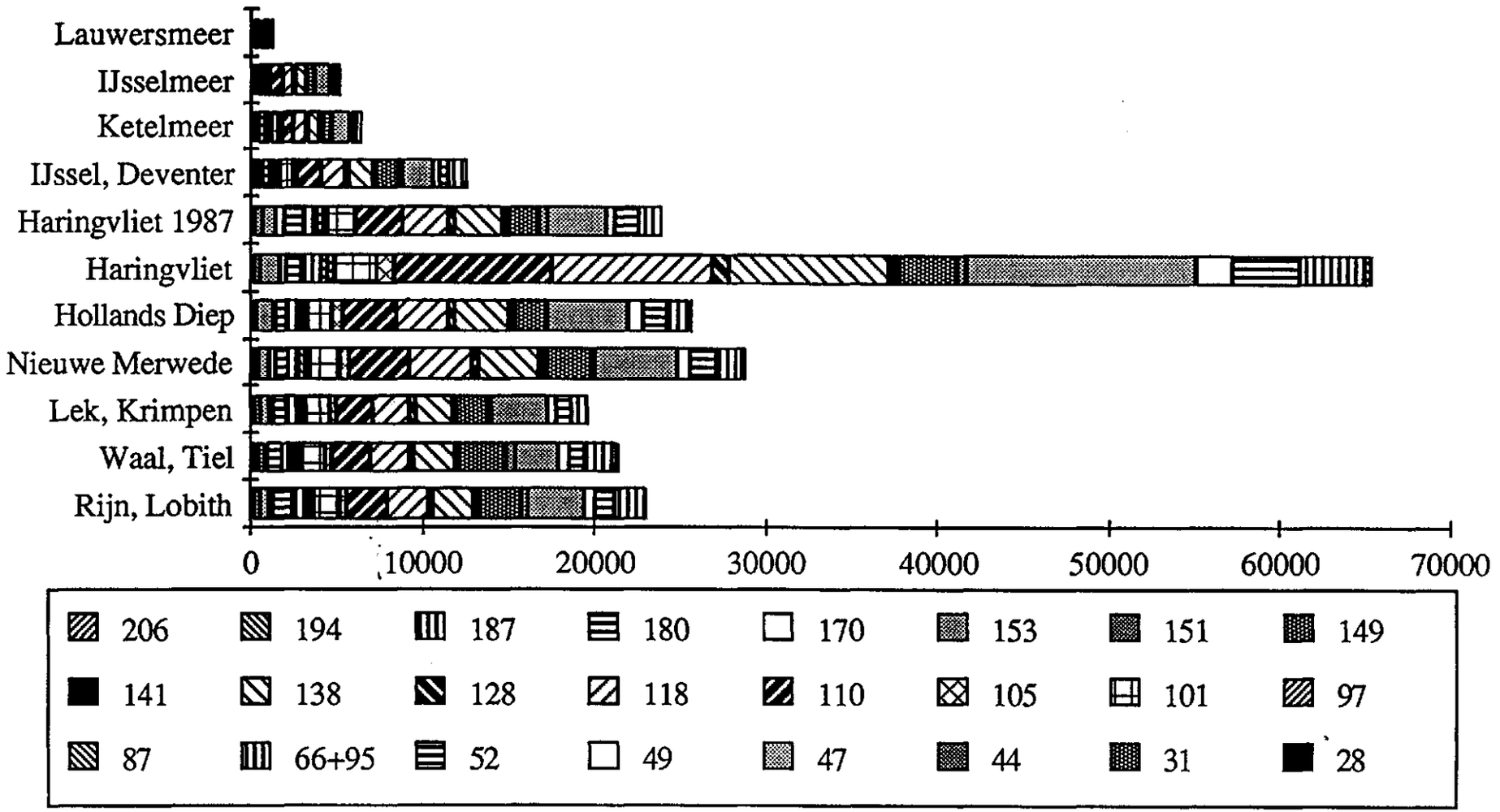

Figure 6a : Contents of individual PCB congeners in yellow eel (µg/kg lipid)

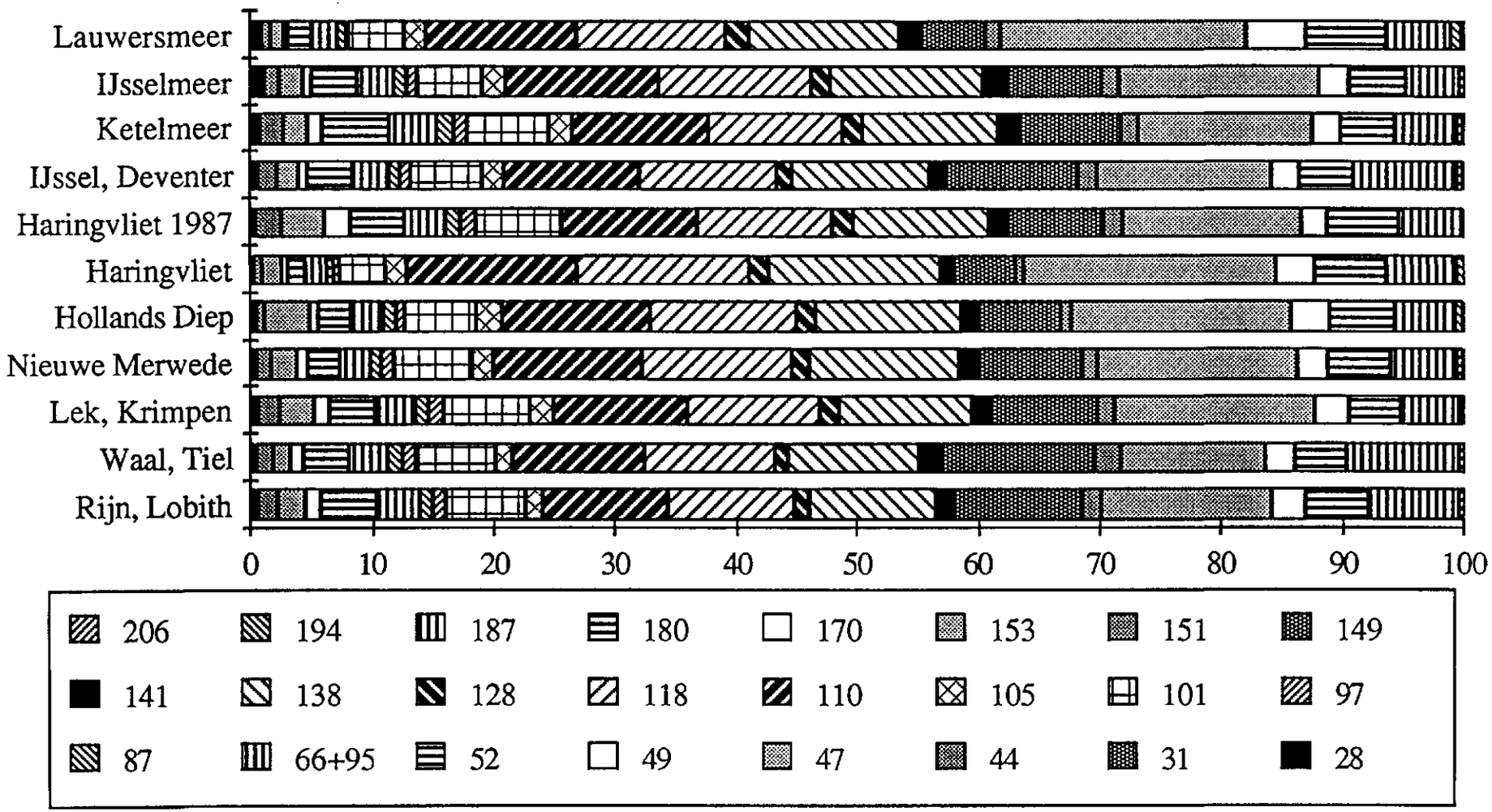

Figure 6b : Relative amounts (%) of individual PCB congeners in yellow eel

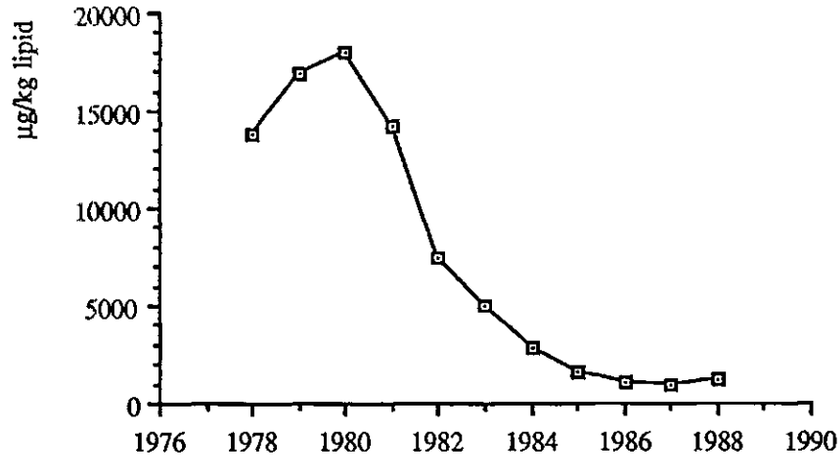

Figure 7a : Trend in HCB contents, on lipid basis, in yellow eel from the Rhine at Lobith

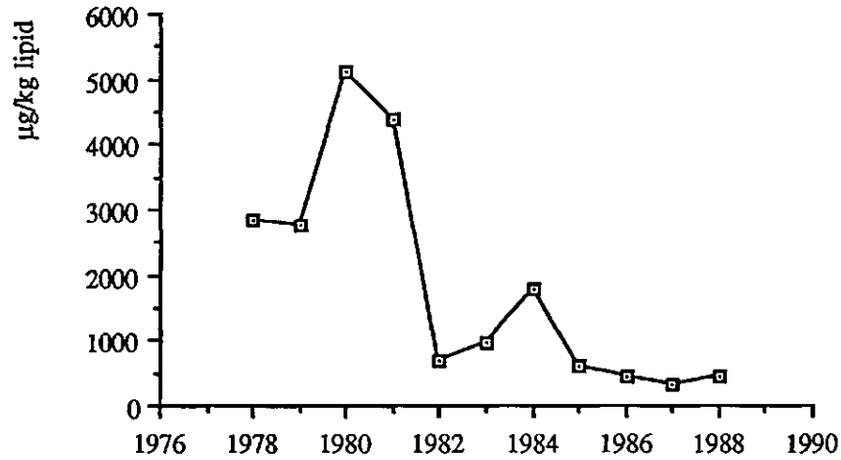

Figure 7b : Trend in QCB contents, on lipid basis, in yellow eel from the Rhine at Lobith

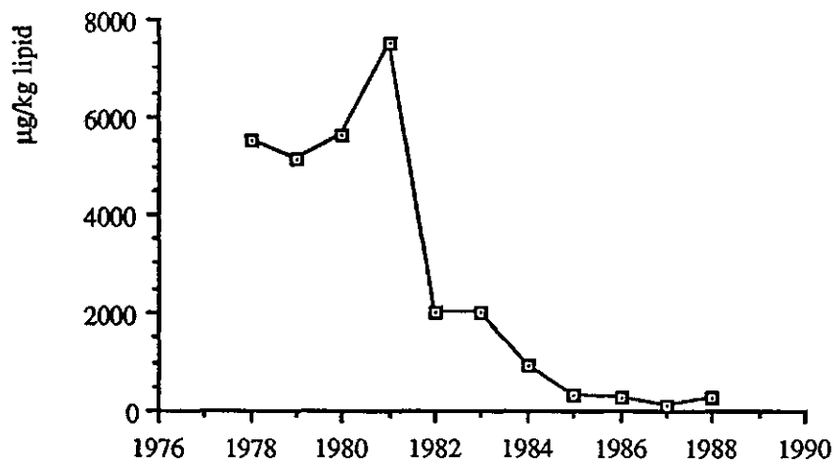

Figure 7c : Trend in HCBD contents, on lipid basis, in yellow eel from the Rhine at Lobith

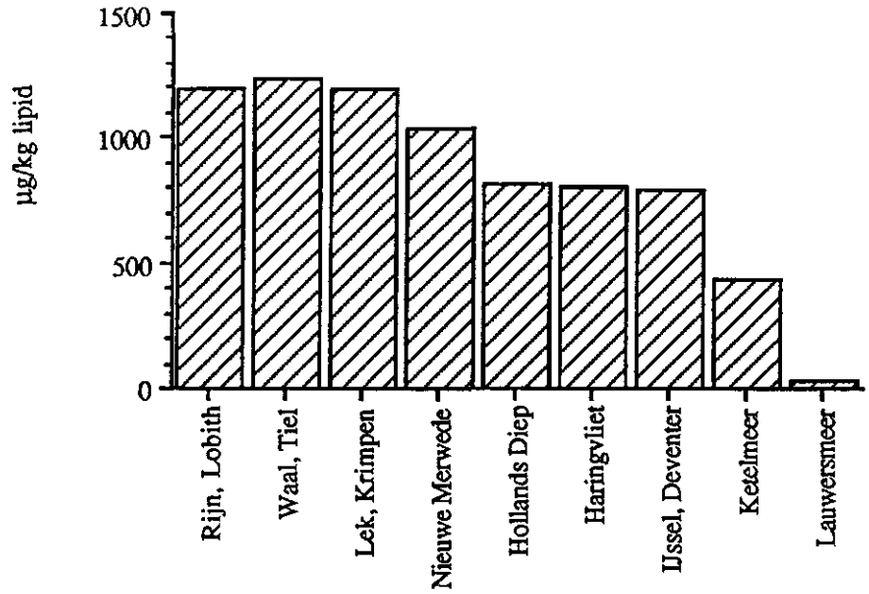

Figure 8a : Observed HCB contents in yellow eel, on lipid basis

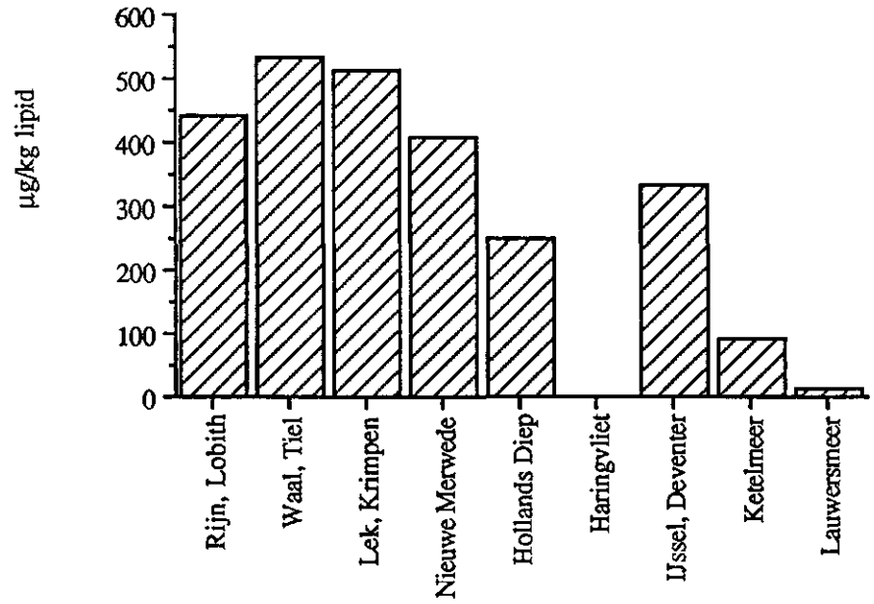

Figure 8b : Observed QCB contents in yellow eel, on lipid basis

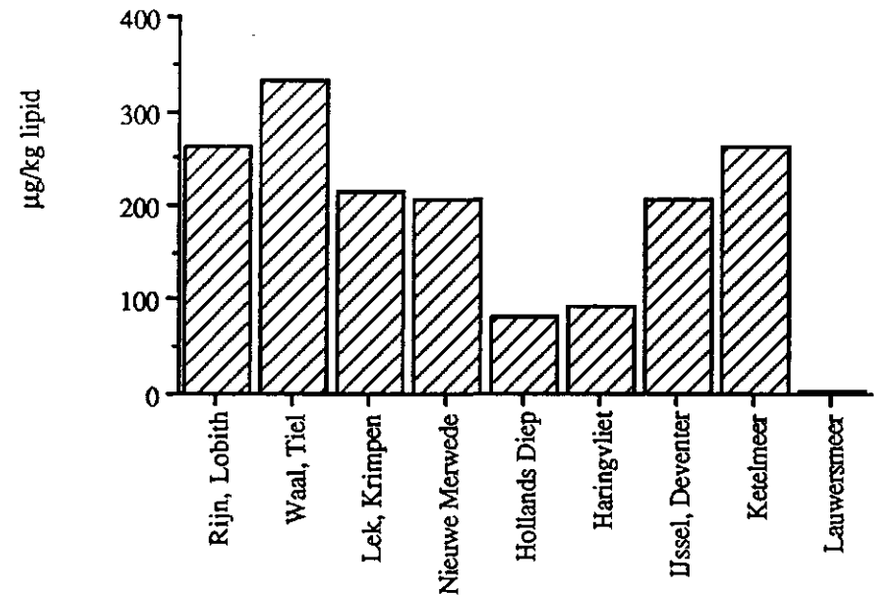

Figure 8c : Observed HCBD contents in yellow eel, on lipid basis

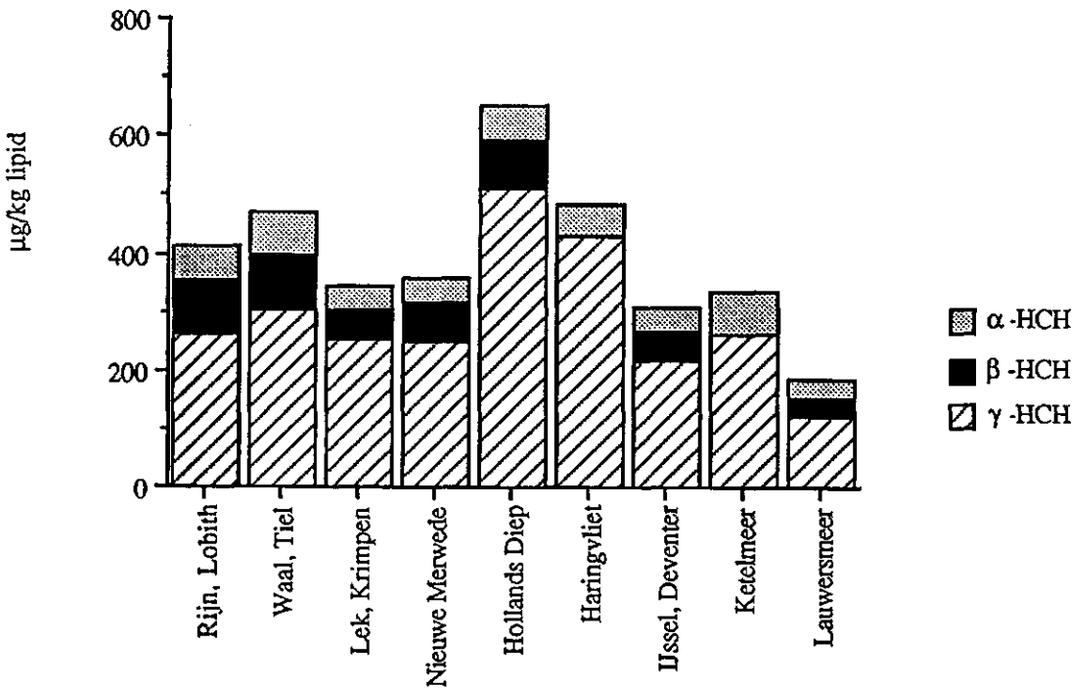

Figure 9 : HCH contents in yellow eel, on lipid basis

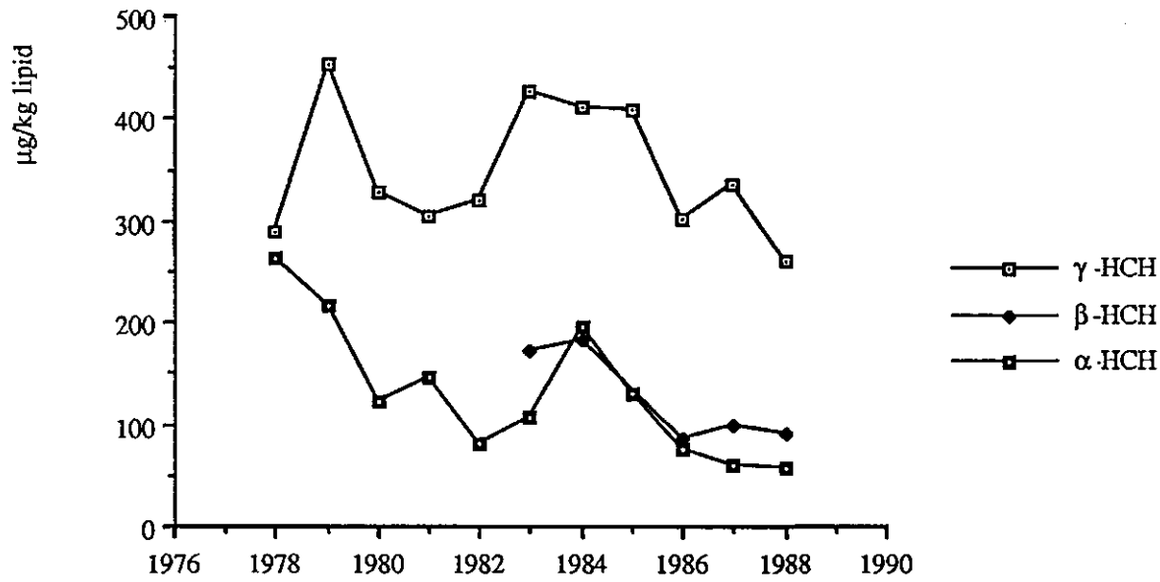

Figure 10 : Trend in HCH contents, on lipid basis, in yellow eel from the Rhine at Lobith

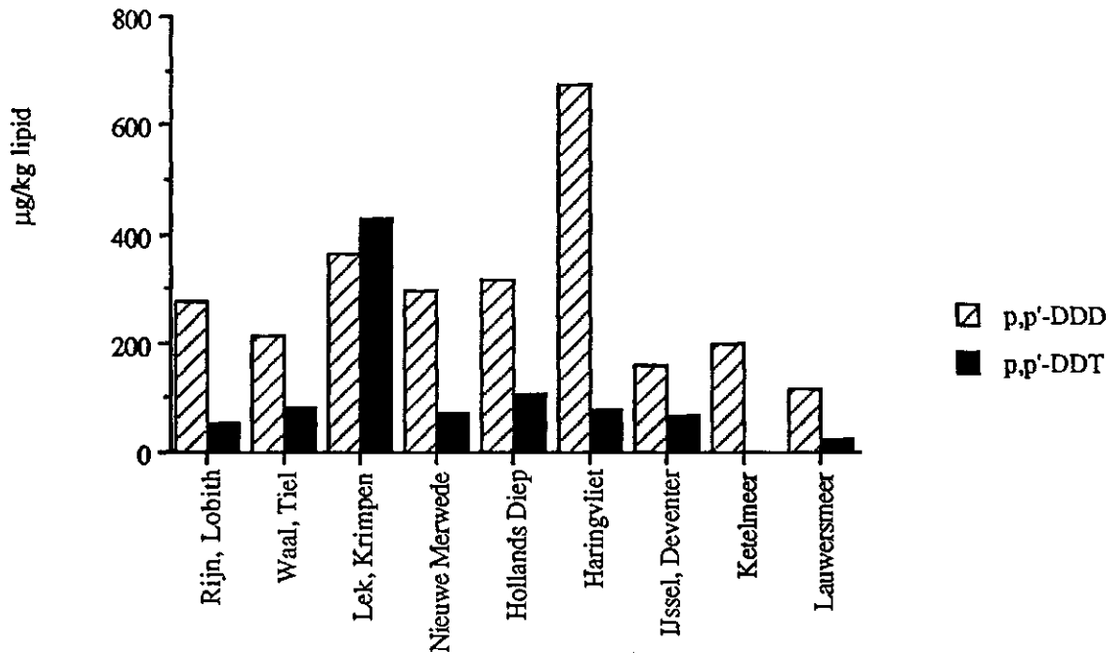

Figure 11a : p,p'-DDD and p,p'-DDT contents in yellow eel, on lipid basis

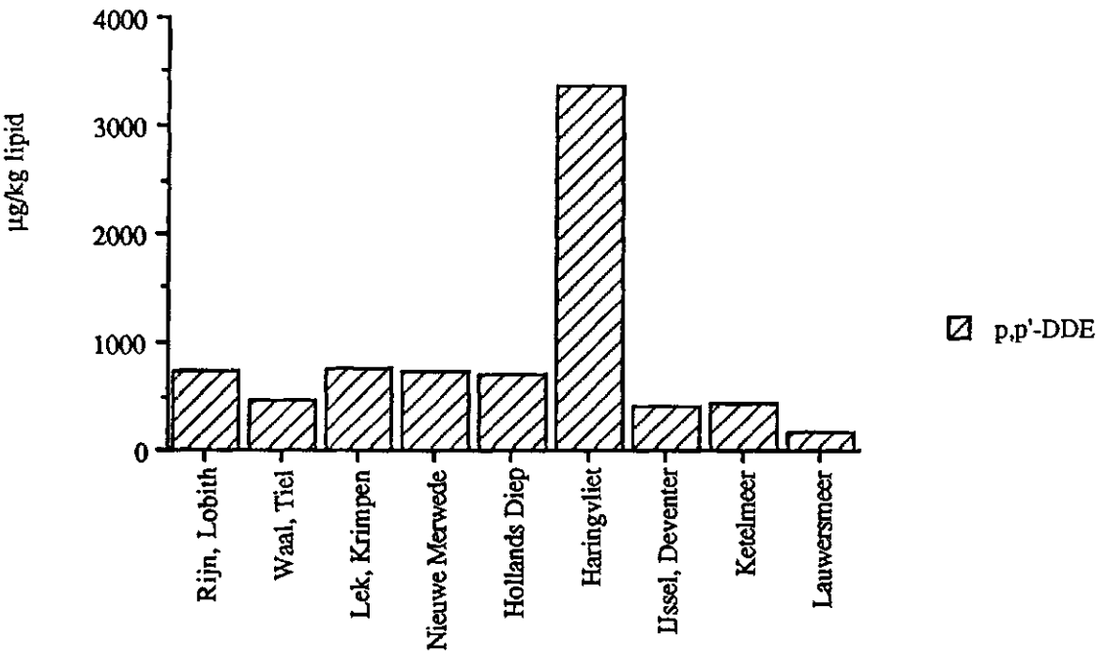

Figure 11b : p,p'-DDE contents in yellow eel, on lipid basis

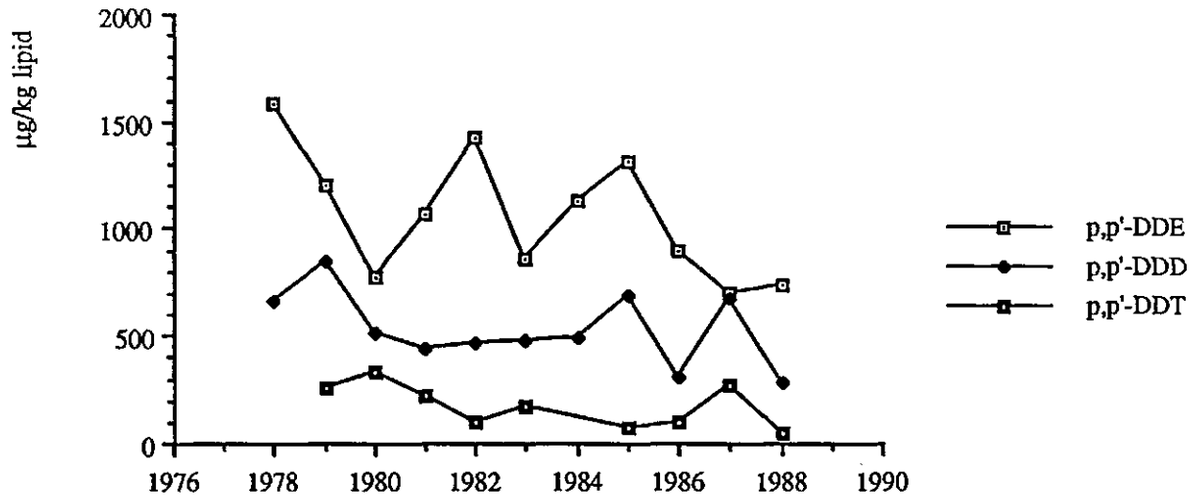

Figure 12a : Time trends for DDT related compounds in yellow eel from the Rhine at Lobith

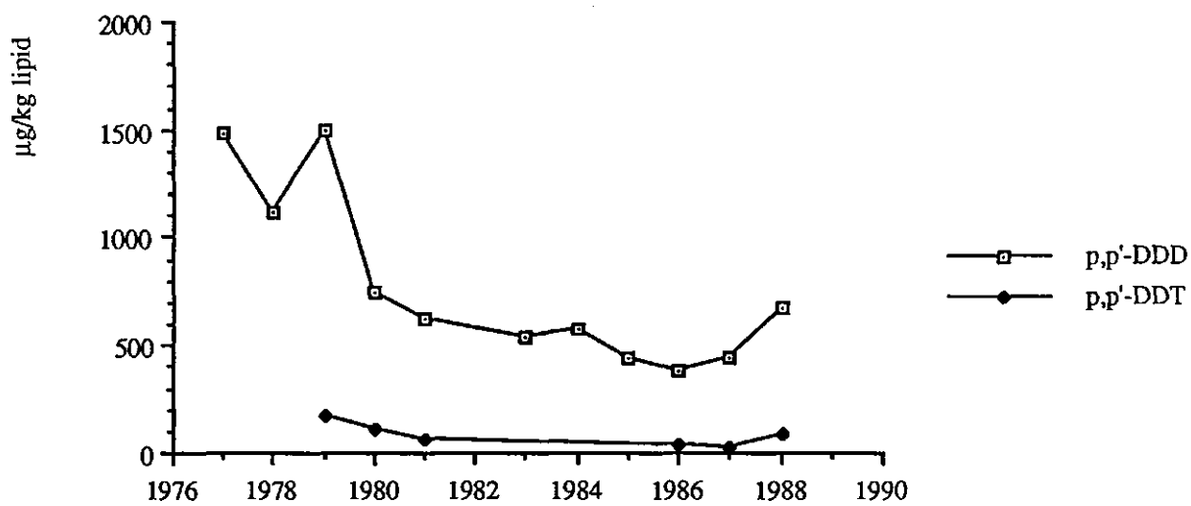

Figure 12b : Time trends for p,p'-DDD and p,p'-DDT in yellow eel from the Haringvliet

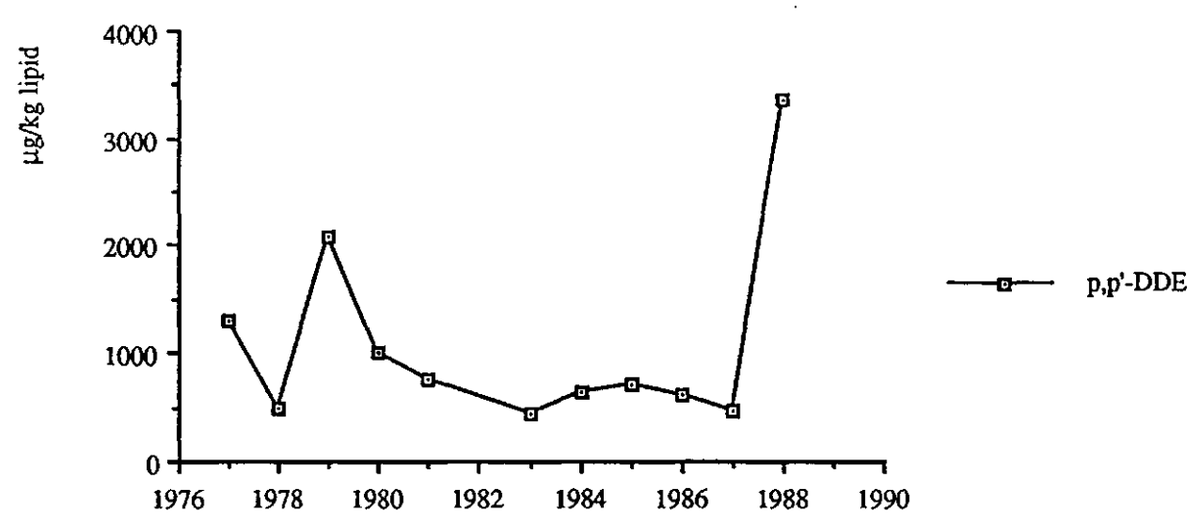

Figure 12c : Time trend for p,p'-DDE in yellow eel from the Haringvliet

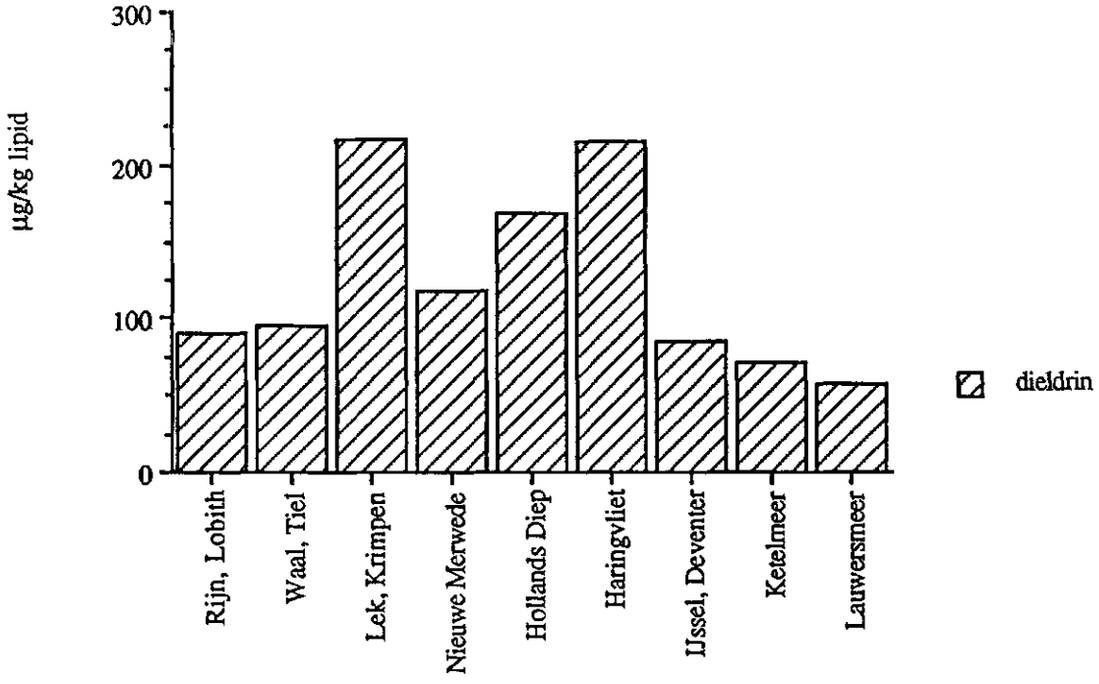

Figure 13 : Dieldrin contents in yellow eel, on lipid basis

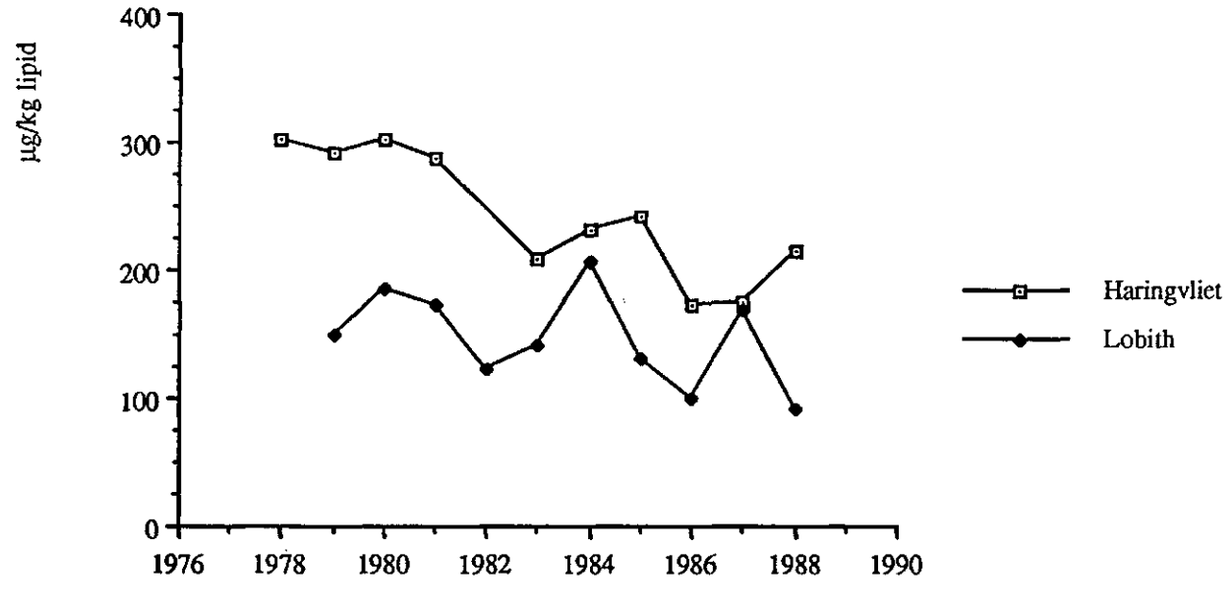

Figure 14 : Trends in dieldrin contents, on lipid basis, in yellow eel

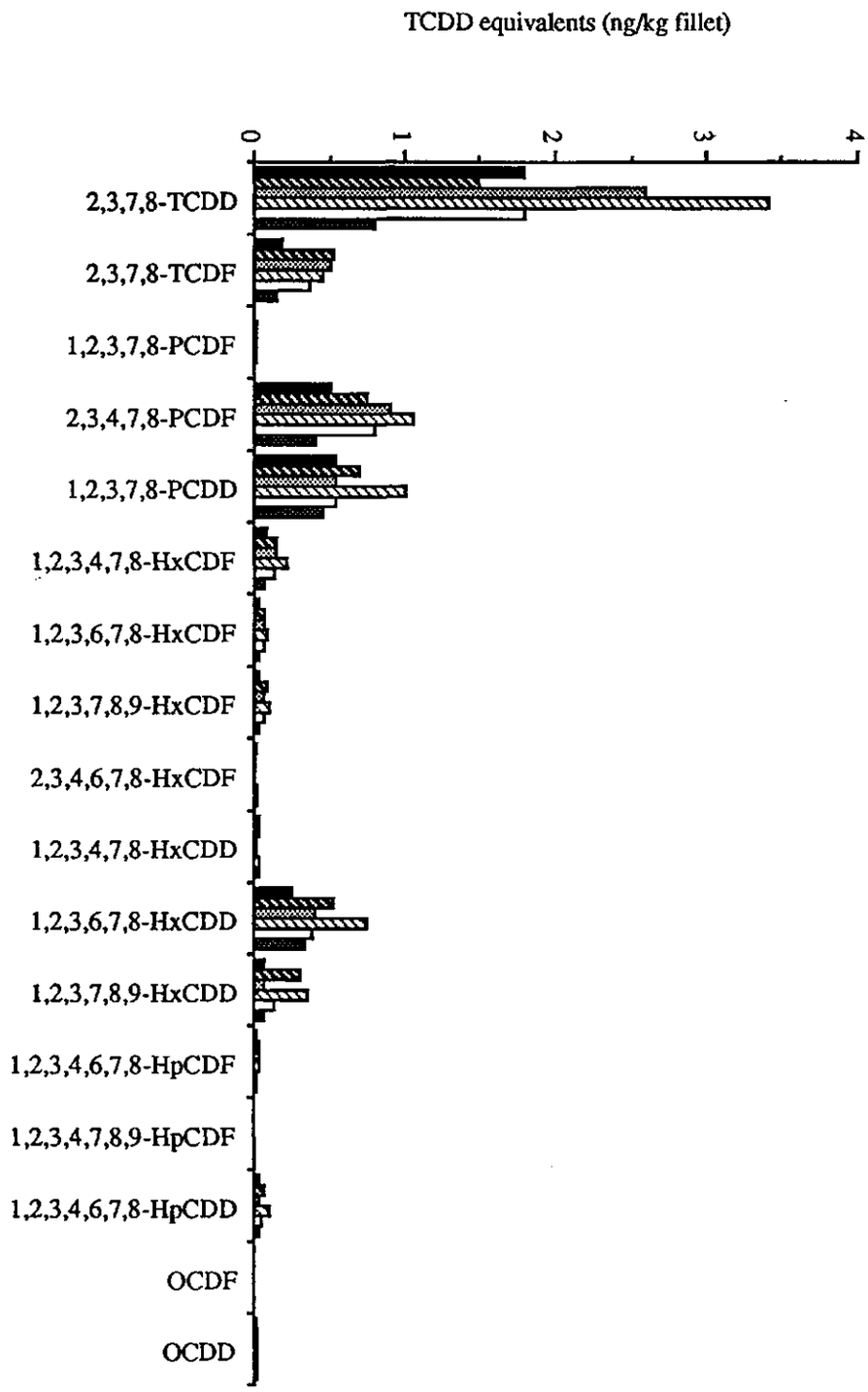

Figure 15 : Individual PCDDs and PCDFs in yellow eel, expressed in TCDD equivalents

Publications and reports of the project "Ecological Rehabilitation of the River Rhine"

no. 1 - 1988 Ecological rehabilitation of the river Rhine: a proposal for a Netherlands research programme.

no. 2 - 1988 Fish and their environment in large european river ecosystems; the Dutch part of the river Rhine. W.G.Cazemier, Science de l'Eau 7, 95-114 (1988).

no. 3 - 1988 High rates of denitrification in a storage reservoir fed with water of the river Rhine. W.Admiraal en J.C.van der Vlugt, Arch.Hydrobiol. 113, 593-605 (1988).

no. 4 - 1988 Impact of biological activity om detritus transported in the lower river Rhine: an excercise in ecosystem analysis. W.Admiraal en B.van Zanten, Freshwater Biology 20, 215-225 (1988).

no. 5 - 1988 Continue signalering van toxische stoffen in het aquatische milieu met behulp van biologische bewakingssystemen - literatuurstudie. J.Botterweg, 31 pp., Den Haag (1988).

no. 6 - 1989 Environmental stress in five aquatic ecosystems in the floodplain of the river Rhine. W.Admiraal, E.D. de Ruyter van Steveninck en H.A.M.de Kruijf. The Science of the Total Environment 78, 59-75 (1988).

no. 7 - 1989 Bioaccumulation in yellow eel (Anguilla anguilla) and perch (Perca fluviatilis) from the Dutch branches of the Rhine-mercury, organochlorine compounds and polycyclic aromatic hydrocarbons. F.van der Valk, H.Pieters en R.C.C.Wegman.