

DIOXINS, FURANS AND DIOXIN-LIKE PCBs IN WILD, FARMED, IMPORTED AND SMOKED EEL FROM THE NETHERLANDS

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Introduction

High levels of dioxins and dl-PCBs in eel from Dutch freshwaters were reported in a screening of Dutch fishery products¹. To protect the Dutch citizens from the intake of high levels of dioxins by the consumption of contaminated eel, a Dutch maximum residue limit (MRL) for eel was set at 8 pg PCDD/F-TEQ/g wet weight (ww)². Following the establishment of this Dutch MRL and anticipating the new European MRL for dioxins and furans in fish (4 pg PCDD/F-TEQ/g ww)³, an extensive survey was carried out on PCB and dioxin contamination of eel from the Netherlands.

Materials and methods

Wild eel

The wild eel survey included 39 freshwater locations originating from main river systems (e.g. Meuse, Rhine), small rivers, canals and lakes (e.g. Lake IJssel). The average length of the eels per sample varied from 33 to 40 cm. For two locations (North Sea Canal and the North Holland Canal) at four moments (resp. week 25, 27, 33 and 38 and week 20, 26, 32 and 37) throughout the catching season (May to October) eel samples were analysed to determine possible variations during the catching season.

Farmed and imported eel

Eleven samples of farmed eel were purchased from Dutch eel farmers. The average length per sample varied from 36 to 56 cm. Fourteen imported eel samples were purchased from different commercial traders (3 wild eel and 11 farmed eel samples). The average length of the eels per sample varied from 39 to 60 cm.

Smoking

The influence of smoking eel on the contaminant levels was determined by smoking 3 batches of eel, either traditionally (n=4) or industrially (n=1) and analysing the raw material and the smoked material.

Analysis

Pooled samples were prepared from fillets of 25 eels per location.

The complete WHO set of dioxins (and furans) and dioxin-like PCBs were analysed in all samples.

The lipid fraction was extracted from the samples by Soxhlet extraction with dichloromethane/n-pentane (1:1 v:v) for 12 hrs. The solvent was evaporated using a rotary evaporator and the lipids were redissolved in ethylacetate/cyclohexane (1:1 v/v) for lipid removal by gel permeation chromatography. The extract was further cleaned by Al₂O₃ column chromatography. The dioxins and non-ortho PCBs

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containing fraction was separated from the other PCBs using a porous graphitised carbon column. The solvent was concentrated to dryness, redissolved in 10 µl toluene and injected on a GC-HRMS system, equipped with a capillary column.

The indicator PCBs and the mono-ortho PCBs were extracted with a mixture of dichloromethane/n-hexane (1:1, v/v) for 6 hrs. The solvent was evaporated using a rotary evaporator and subsequently the lipids were removed by Al₂O₃ chromatography. After concentration of the eluate, the extract was further cleaned using silica chromatography. After treatment of the extract with concentrated sulphuric acid the extracts were analysed using a HP-6890 Hewlett-Packard GC with splitless injection (250 °C) and electron capture detection (ECD). The capillary column used was a CP-Sil-8 (50m, 0.15 mm i.d., 0.30 µm film thickness).

The total-TEQ levels in all samples were also determined by application of a CALUX bioassay. The extracted fat was dissolved in n-hexane and pre-cleaned using acidified silica chromatography. The solvent was concentrated to 20 µl and the cleaned extract was redissolved in dimethylsulfoxide (DMSO) and applied to the bioassay. After incubation (20 h, 37 °C), the cells were lysed and after addition of a reagent the luciferase was detected in the lysate using a luminodetector. The fat content was determined gravimetrically.

Results and discussion

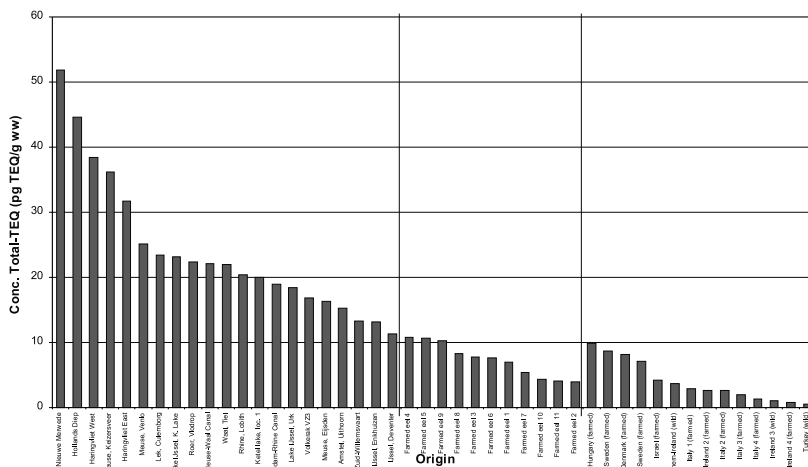


Figure 1. Total-TEQ levels (pg total-TEQ/g ww) for wild eel samples, farmed eel and imported eel samples. For wild eel samples only levels >10 pg total-TEQ/g ww are shown.

Figure 1 and Table 1 show a broad contamination range (ca. 50-fold difference between the lowest and the highest values) for dl-PCBs and dioxins in wild eel. The most heavily contaminated eel samples originate from the main rivers which are subjected to a high degree of industrialisation. Due to the high fat content of eel and the high degree of pollution of the eel's habitat, the levels in these eel samples are significantly higher than the levels normally observed in other fish^{1,4,5}.

During the catching season (May to October) the levels of contaminants on two fresh water locations varied by a factor of ca. 2 on a wet weight basis, most likely because eel increased their lipid-depot for hibernation (data not shown). On a lipid weight basis the variation was much smaller. The ranges of the farmed and imported eel are much smaller and concentrations are significantly lower than

those observed in the majority of the wild eel samples. The most important factor contributing to the levels in farmed eel is the origin of the fish oil and fish meal used for feeding the eels⁶. In wild eel, the levels of dioxins ranged from 0.3 to 7.9 pg TEQ/g ww which is just below the current Dutch dioxin tolerance level of 8 pg TEQ/g ww. However, 7 out of 39 wild eel samples exceeded the EC dioxin MRL of 4 pg PCDD/F-TEQ/g ww. This shows that wild eel from at least part of the current fishing areas in the Netherlands is no longer fit for human consumption. This will be valid for more areas when dioxin-like PCBs will be included in a possible future European tolerance level for dioxins and PCBs as the dl-PCBs contribute for 61-97 % to the total-TEQ. From this point of view, the current EU MRL which only accounts for the dioxins does not satisfy for the protection of consumers concerning these highly contaminated eel samples.

Table 1. Levels of dioxins, dl-PCBs and the sum of the indicator PCBs and in eel samples from the Netherlands ($\mu\text{g}/\text{kg}$ ww).

Samples	n	GC-HRMS			CALUX	GC-ECD
		dl-PCBs	PCDD/Fs (pg TEQ/g ww)	Total-TEQ ²	Total-TEQ ² (pg TEQ/g ww)	Sum 7 PCBs ¹ (ng/g ww)
Freshwater eel	39	0.8-44	0.3-7.9	1.0-52	0.89-52	13-1740
Farmed eel	11	3.9-7.7	0.9-3.1	3.8-11	1.9-15	21-58
Imported eel	14	0.3-6.8	0.2-3.0	0.5-9.8	0.2-20	<1.1-65

¹ indicator PCBs: Nos. 28, 52, 101, 118, 138, 153 and 180

² Sum of dioxins and dioxin-like (non-ortho and mono-ortho) PCBs

Comparison of the total-TEQ data based on the congener specific GC-HRMS analysis with the total-TEQ data and the CALUX-bioassay shows that, in general, the CALUX data show higher levels compared with GC-HRMS data (on average 114, 161 and 143% for wild, farmed and imported eel respectively). However, in some cases CALUX-bioassay data is lower than the GC-HRMS data. The differences between both methods are due to interference's that influence the response of the bioassay. Considering future use of the CALUX-bioassay for screening purposes, care should be taken (by safety margins) to avoid false negative results.

Regarding the indicator PCBs, the results show high concentrations, up to 1.7 $\mu\text{g}/\text{g}$ ww. The highest concentrations are observed in the same rivers which show high dioxin and dl-PCB concentration. Small rivers, canals and lakes which are not directly influenced by industries show much lower levels, generally below 10 $\mu\text{g}/\text{kg}$ ww. Eels from three locations (Hollands Diep, Nieuwe Merwede (both river Rhine delta) and the river Meuse) exceed the Dutch tolerance level for CB-153 (500 $\mu\text{g}/\text{kg}$ ww)⁷. The levels of PCBs in farmed and imported eel are relatively low compared with wild eel.

The results of the smoking experiments in Table 2 do not show dramatic level changes for the contaminants for the smoked eel samples (except the imported eel). Increased levels for the total-TEQ (+25 %) are found as well as decreased levels (max. -15 %) for both farmed eel samples. The reason of the dramatically increased levels of the smoked imported eel is unclear. Literature data shows average decreases of 30-39 % of PCBs and total-DDT after smoking⁸. However, in these studies only lean fishes are smoked, with a different distribution of the lipids compared with eel. This makes comparison of data difficult.

A significant decrease of 60-95 % is found of the lower chlorinated PCBs (CB-28, 31, 44, 47, 49 and 52; data not shown) which is most likely due to the evaporation of these PCBs from the eel during smoking of the eel sample.

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Table 2. Changes in levels of dioxins, dioxin-like PCBs and the indicator PCBs due to smoking

Sample	Smoking process	Moist. (%)	Lipid (%)	PCDD/Fs (pg TEQ/g ww)	dl-PCBs (pg TEQ/g ww)	Total-TEQ (pg TEQ/g ww)
Farmed eel 1	Starting material	47	37	1.8	5.1	6.9
	Industrial	39	42	1.7	5.1	6.8
	Traditional ²	38	44	2.2	6.4	8.6
Farmed eel 2	Starting material	49	35	0.90	3.0	3.9
	Traditional ¹	46	35	0.89	2.8	3.6
	Traditional ²	47	34	0.67	2.6	3.3
Imported eel	Starting material	61	20	0.22	0.6	0.82
	Traditional ²	56	19	0.53	1.8	2.3

¹ The eel was smoked by the supplier of the eel

² The eel was smoked by the same smoker

Conclusions

Dioxin, dl-PCBs and indicator-PCB concentrations in Dutch eel from several freshwater locations are relatively high compared with literature data. Levels in farmed and imported eel are significantly lower than those in the highest wild eel samples. Within one season levels (on wet weight basis) can vary by a factor ca. 2. Seven wild eel samples exceed the EU MRL of 4 pg-PCDD/F-TEQ/g ww. None of the farmed and imported eel (mostly farmed) exceed the EU MRL.

The current MRL only accounts for the PCDD/F-TEQ whereas for these samples PCBs are the main contributing compounds (61-97 %) to the total dioxin toxicity (total-TEQ). In this respect, the EU MRL does not satisfy for protection of consumers.

Smoking of eel does not necessarily decrease the levels of dioxins and dl-PCBs. Both decrease and an increase of total-TEQ levels by 15 and 25 % respectively, are observed. Due to their volatility, some of the lower chlorinated PCBs (up to tetra-CB) are decreased in all samples by 60-95 % as a result of heating the eel during the smoking process.

The CALUX bioassay can be useful for screening total-TEQ values in eel samples, in respect of the EC MRL. However, due to interference's, safety margins should be taken into account to avoid false negative results.

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