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Persistent organic pollutants (POPs) in fish collected from the urban tract of the river Tiber in Rome (Italy)

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Summary. European eel and chub samples were analyzed to determine the levels of non-dioxin-like polychlorobiphenyls (NDL-PCBs), polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs), dioxin-like PCBs (DL-PCBs), and brominated polybromodiphenyl ethers (PBDEs) in order to evaluate the extent of contamination of the river Tiber along the urban tract through the city of Rome (Italy). All samples presented detectable levels of the chemicals analyzed, and exhibited species-specific differences in terms of congener composition and total concentrations. On average the European eel presented the highest values. In this species the dioxin-like compound sums (WHO-TEQs) exceeded the pertinent maximum levels (MLs). Non-*ortho* PCBs constituted approximately 80% of WHO-TEQ toxicological potential whereas NDL-PCB and PBDE concentrations appeared to match values determined in other polluted aquatic ecosystems where non-point contamination sources were present. The contamination patterns determined in fish tissues seemed to reflect the impact of generic contamination source(s).

Key words: eel, chub, persistent organic pollutants, river Tiber.

Riassunto *(Contaminanti organici persistenti in pesci raccolti nel tratto urbano e nel fiume Tevere a Roma)*. In questo studio sono stati analizzati esemplari di anguilla europea e cavedano per la determinazione dei livelli di policlorobifenili ad azione non diossina-simile (NDL-PCBs), policloridibenzo-p-diossina (PCDDs) e policlorodibenzofurani (PCDFs), PCB ad azione diossina-simile (DL-PCB), difenil eteri bromurati (PBDE), al fine di valutare la contaminazione del fiume Tevere lungo il tratto urbano della città di Roma. Tutti i campioni presentano livelli distinguibili delle sostanze chimiche analizzate, ed esibiscono differenze specie-specifiche in termini di composizione di congeneri e livelli totali. In media, l'anguilla europea presenta i valori più elevati. In questa specie la somma dei composti ad azione diossina-simile supera il livello massimo previsto dalla normativa comunitaria. I PCBs non-orto costituiscono approssimativamente l'80% del potenziale tossicologico.

Parole chiave: anguilla, cavedano, contaminanti organici persistenti, fiume Tevere.

INTRODUCTION

POPs are substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment [1]. These are widespread and, as a result of transport mechanisms from site of their use, they have been detected even in remote locations (*e.g.* the Arctic area). Actually, the impact due to traditional pollutants such as polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), etc., appears to have decreased in relation to the implementation of international agreements such as the Stockholm Convention [1]. However, industrial and urban settlements continue to be a source of such chemicals and other consumer compounds such as PBDEs [2, 3]. In turn, these chemicals can influence urban biodiversity by their toxic action and by the interaction with anthropogenic physical impacts [4, 5] which appear to be of major concern in an urban context.

In order to understand the potential impact of these chemicals in the urban tract (City of Rome) of the river Tiber, we considered two indicator species such as the eel *(Anguilla anguilla)* and the chub *(Leuciscus cephalus)*, with different ecological niches and collected from the same river tracts. Eel, in particular, is a popular bioindicator of POP contamination [6].

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The concentrations of non dioxin-like PCBs (NDL-PCBs), PCDDs, PCDFs, dioxin-like PCBs (DL-PCBs), and polybromodiphenyl ethers (PBDEs) in muscle tissues from the two fish species are presented and discussed. As these fish are consumed by selected population groups, the results are evaluated in the light of EC Regulation 1881/2006, setting maximum levels (MLs), and EC Recommendation 88/2006, setting action levels (ALs). To our knowledge, little effort has been made until now to investigate concentrations of priority POPs in fish from the Italian rivers. The study of these concentrations has an importance that goes beyond the evaluation of the urban impact as fish from (urban) rivers are part of some people's diet.

MATERIALS AND METHODS

For the purpose of the study, the European chub was chosen as a representative of species living in the water column. Its behavior is quite different from that of the eel: they exhibit diverse degrees of mobility and habitats, the latter differing from an ecological point of view. The European chub is considered to be nomadic and rheophile (*i.e.* preferring flowing waters) and is omnivorous. The European eel does not spawn during their residence in European waters and are benthic predators. Further, eels accumulate organic microcontaminants in their muscle tissue readily as it contains a high proportion of fat. For this reason eels are well suited for use in monitoring programs. Eel are also a popular foodstuff and therefore represent a potential health hazard to some consumer groups.

Fish specimens were taken from three sites along the urban tract of the river Tiber in Rome, respectively called Ponte Milvio, Trastevere, and Magliana. Fish were caught in the summer using fishing nets, usually in slow flowing reaches. Where possible, fish of similar size were selected for the analyses, their body length and weight were recorded, and the bodies pooled together. For the eel and chub specimens, the average weights and lengths were, respectively, 142 g and 35 cm, and 285 g and 32 cm.

Upon delivery to the analytical laboratory, the specimens collected were stored at -20 °C until pretreatment. For pretreatment, specimens were allowed to thaw; and individually rinsed with distilled water. Fish matrices for analysis were made of skinned fillets taken from several individual specimens and homogenized.

Thirty NDL-PCBs, the 17 PCDD and PCDF congeners, eight mono-*ortho* substituted DL-PCBs, non-*ortho*-substituted DL-PCBs 77, 81, 126, and 169, and the PBDEs 17, 28, 47, 66, 99, 100, 138, 153, 154, and 183 were determined. The analytical procedure was adapted from the US EPA Method No. 1613 [7] for PCDD and PCDF determination.

Muscle samples of about 20 g were added with ^{13}C-labelled standards (ISs), allowed to rest for hours, homogenized with anhydrous Na_2SO_4, and extracted for nine hours using a Soxhlet apparatus with a 50% mixture of acetone and n-exhane. Each extract was concentrated to 20 mL using a rotary evaporator; a 2 mL aliquot was used for lipid content determination by gravimetric method. Clean-up was carried out by filtration through Extrelut impregnated with concentrated sulphuric acid and a silica gel layer [8]. The extract was analyzed for PBDEs, PCDDs, PCDFs, and non-*ortho*-substituted DL-PCBs with three different Power-Prep separation programs whereas mono-*ortho*-substituted DL-PCBs and NDL-PCBs were determined directly without any further clean-up step.

Quantification was performed by high-resolution gas chromatography coupled with low-resolution mass spectrometry (HRGC-LRMS) used in the selected ion monitoring mode (SIM) for NDL-PCBs and PBDEs. HRGC-HRMS(SIM) was employed to determine PCDDs, PCDFs, and DL-PCBs operating at 10 000 mass resolution. A procedural blank was run together with three to five samples. Reliable measurements were allowed above the limit of determination with a repeatability in the order of |±10 %| (extended uncertainty, |±20 %|). The recovery rates of ISs were accepted within 40-120 %; values outside this range led to specific evaluation, possibly rejection of trial.

RESULTS AND DISCUSSION

Non-dioxin-like polychlorobiphenyls (NDL-PCBs)

Measured PCB concentrations are reported in *Table 1* as the sums of the thirty PCB congeners determined (Σ_{30}) and of the six (Σ_6) and seven (Σ_7) indicator congeners. All the concentrations found in the eel are higher than the those in the chub. However, except for the chub specimens collected at Ponte Milvio and Trastevere, the data are in the same order of magnitude. In particular, the ranges based on the determination of 30 NDL-PCB congeners are 245-427 ng/g wet weight *(ww)* for the eels, and 49.4-150 ng/g *ww* for the chub. The Σ_7 and Σ_6 concentrations follow the same patterns exhibited by Σ_{30} and can be compared with the Σ_6 average concentration of 12.5 ng/g *ww* reported by the European Food Safety Authority [9]. As can be seen in the table, all the Σ_6 levels detected in the eel and chub specimens exceed the above-mentioned level up to more than one order of magnitude.

In the Flanders, the eel is used as an indicator of environmental and potential exposure to the chemicals distributed in waters and sediments [6]. In the aforesaid paper, no specific exposure sources were identified and the data derived from an extensive *ad hoc* monitoring activity. In this context, an average Σ_7 concentration of 605 ng/g *ww* was found in eels of approximately the same average size as our specimens. This value is about two times the values determined in our study.

From the profiles of *Figure 1* the main differences between the two species can be seen at the levels of the tri- and tetra-chlorosubstituted NDL-PCBs (PCBs 18, 33, 49, and 70). From a qualitative point of view, their levels appear to be consistently higher in chub than in eel. This tendency has been observed in another study where the European eel and the com-

Table 1 | *Contamination levels detected in eel and chub specimens collected from the urban tract of the river Tiber in Rome, Italy. All concentrations are given on a fresh weight basis (fw). Cumulative analytical concentrations (Σ) are medium bound estimates. Cumulative concentrations are medium bound estimates. Values rounded off to three figures.*

	EEL *(Anguilla anguilla)*			CHUB *(Leuciscus cephalus)*		
	P. Milvio	Trastevere	Magliana	P. Milvio	Trastevere	Magliana
Σ_{30} ngPCB/g	427	383	245	49.4	97.8	150
Σ_{6} ngPCB/g	335	220	148	34.5	75.6	124
Σ_{7} ngPCB/g	372	264	167	38.6	83.9	138
Σ_{13} ngPBDE/g	33.8	45.5	29.5	7.53	14.9	17.9
Σ_{17} pg PCDD+PCDF/g	5.44	8.53	4.41	4.10	4.04	2.90
Σ_{7} pg PCDD/g	2.33	4.35	2.50	0.60	0.66	0.90
Σ_{10} pg PCDF/g	3.12	4.18	1.91	2.31	3.38	3.20
pg WHO-TE/g PCDD+PCDF	1.00	1.99	0.901	0.460	0.770	0.740
pg WHO-TE/g non-ortho DLPCBs	5.21	8.63	4.07	1.23	2.29	3.77
pg WHO-TE/g mono-ortho DLPCBs	11.3	10.7	6.81	4.69	2.51	1.19
pg WHO-TE/g DL-PCBs	16.5	19.3	10.9	5.92	4.88	4.95
TOTAL TEQs/g	17.5	21.3	11.8	6.38	5.57	5.69

mon trout were comparatively used as bioaccumulation bioindicators in the river Turia [10]. Therefore, the tri- and tetra-chlorosubstituted NDL-PCBs appear to characterize PCB profiles determined in fish living in the water column, the ones more susceptible to bioconcentration [11], for their relative by higher water solubility.

Polybromodiphenyl ethers (PBDEs)

Total PBDE concentrations found in eel and chub are show in *Table 1*. The ranges determined in both species are 29.54-45.5 ng/g *ww* and 7.53-17.9 ng/g *ww*, respectively. The lowest values were determined, as expected, for the chub. This difference, already observed for PCBs, is due to a different lipid contents between the two fish. Additionally, the species-specific distribution of the compounds *(Figure 1)* shows simpler patterns in chub than in eel with more congeners close to their determination limits *(DL)*.

A total PBDE concentration estimate of 18 ng/g *ww* was found in carps collected in the Flanders, Belgium [12], from sites not exposed to point contamination sources. In a survey of US Great Lakes, 65 ng/g *ww* of total PBDEs were found in carps from a river near a manufacturing facility [13]. In Norway, the PBDE concentration range found in fillets of brown trout collected from various lakes was 0.3-40 ng/g *ww*, whereas in specimens collected from a lake under impact from a textile manufacturing facility the range was 156-2265 ng/g *ww* [14]. According to our data, in both species, the most abundant congener is PBDE 47 followed by PBDE 100; some differences can be observed for the other congener levels *(Figure 1)*. In particular, in the eel specimens congeners 49, 99, 153, and 154 were detected at appreciable levels whereas in the chub only PBDE 28 and 154 appear to have some relevance. In general, the predominance of PBDE 47 in the fish tissues is largely confirmed by other studies with different fish species such as carps [12, 15] and brown trout [14]. On the whole, despite the above-mentioned species-specific differences, the congeners detected make up an average contamination profiles to which non-point sources are likely to be the main contributors. This has been shown in a large survey carried out in Virginia rivers [16] and in the mentioned studies of various lakes in the south-eastern part of Norway [14] and of the north American Great Lakes area, except where a specific source could be identified [13, 14]. In particular, according to the last two studies the non-point source profile seems to reflect the contribute of air concentrations.

Dioxin-like compounds (PCDDs, PCDFs, and DL-PCBs)

The analytical and WHO-TEQ concentrations of PCDDs and PCDFs are summarized in *Table 1*. In toxicity equivalents, the concentrations estimated in eel and chub specimens range 0.901-1.99 pgWHO-TE/g *ww* and 0.466-0.770 pgWHO-TE/g *ww*, respectively. As observed for NDL-PCBs and PBDEs, eels show higher contamination levels, 2-4 times greater than those measured in chub due to its highest lipid content. Additionally, the ratios $[PCDFs]\times[PCDDs]^{-1}$ show different characteristics between the two species: the PCDD component is higher than the PCDF component in the eel, whereas in the chub the ratio $[PCDFs]/[PCDDs]^{-1}$= 2.3-3.1 reflects a higher contribution from PCDFs.

The profiles presented by the eel specimens *(Figure 1)* show visible differences among the three specimens than those showed by the chub; in the chub a profile consistency is visible among the specimens with many congeners close to their determination limits. In the

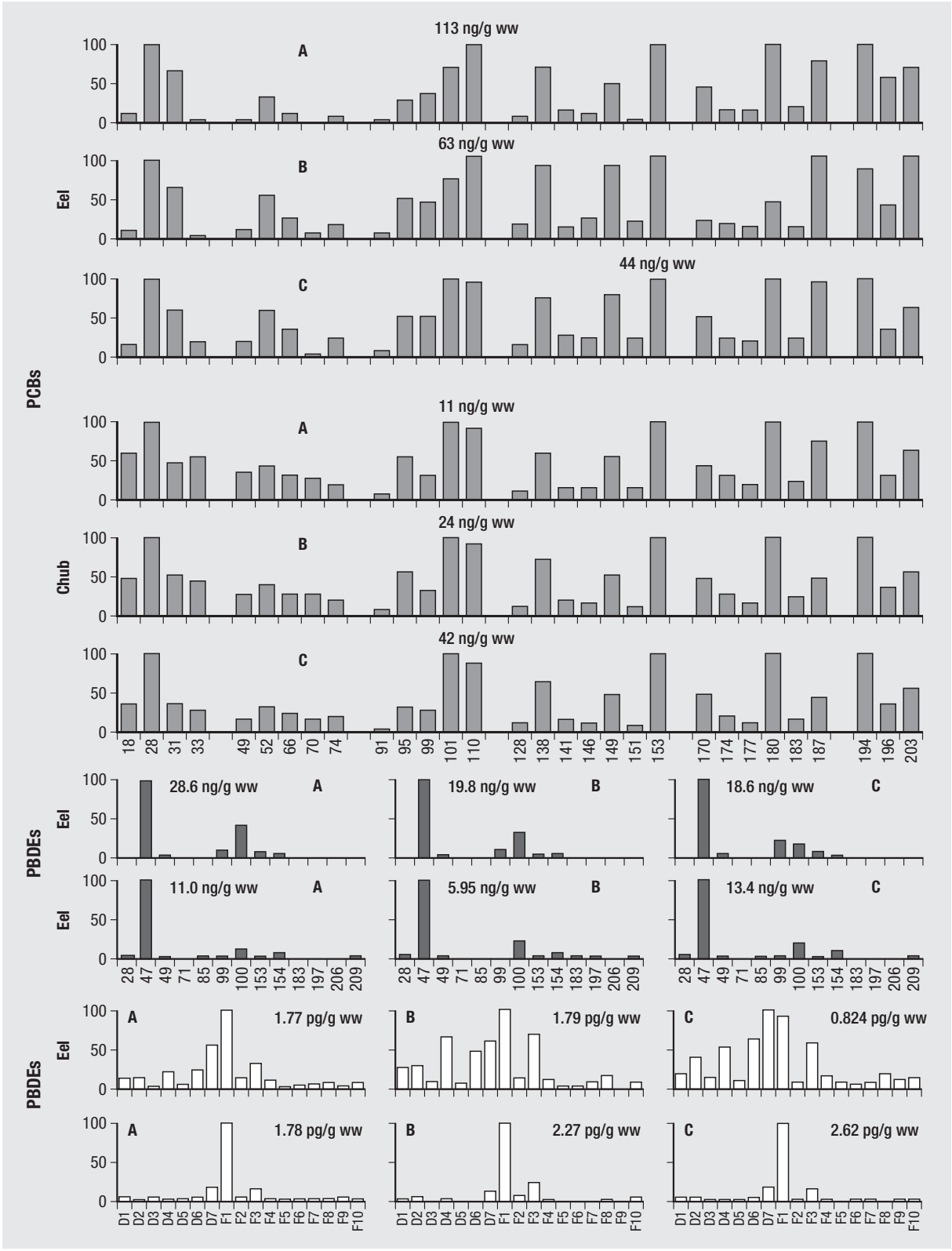

Fig. 1 | *NDL-PCB, PBDE, and PCDD and PCDF analytical congener profiles detected in eel and chub specimens collected from the urban tract of the river Tiber in Rome, Italy. The three sampling sites are (a) Ponte Milvio, (b) Trastevere, and (c) Magliana. For NDL-PCBs the graphs report the 30 determined subdivided according to the degree of chlorosubstitution. White bars indicate limits of determination. aD1, 2,3,7,8-T4CDD; D2, 1,2,3,7,8-P5CDD; D3, 1,2,3,4,7,8-H6CDD; D4, 1,2,3,6,7,8-H6CDD; D5, 1,2,3,7,8,9-H6CDD; D6, 1,2,3,4,6,7,8-H7CDD; D7, O8CDD; F1, 2,3,7,8-T4CDF; F2, 1,2,3,7,8-P5CDF; F3, 2,3,4,7,8-P5CDF; F4, 1,2,3,4,7,8-H6CDF; F5, 1,2,3,6,7,8-H6CDF; F6, 1,2,3,7,8,9-H6CDF; F7, 2,3,4,6,7,8-H6CDF; F8, 1,2,3,4,6,7,8-H7CDF; F9, 1,2,3,4,7,8,9-H7CDF; F10, O8CDF.*

eel, the profiles corresponding to the Trastevere and Magliana sites exhibit some overlapping whereas the Ponte Milvio specimen displays other features, suggesting a differential impact on the three study areas. The predominant congeners in specimens collected from the Trastevere and Magliana sites are F1 and D7 followed by the congeners D6, F3, D4, D2, D1, exhibiting comparable heights *(Figure 1)*. In the Ponte Milvio specimen only the 2,3,7,8-TCDF and the OCDD appear to be remarkable, whilst the other congeners are significantly below the corresponding ones in the above-mentioned profiles. In the chub specimens only the F1 shows a relevant presence. These remarkable inter-specific difference could be attributed in part to the different ecological niches. However, though there is a need of further studies to elucidate the species-specific metabolic role in determining such differences.

The DL-PCBs contamination reflects the general pattern of contamination found in European fish [17]. In particular, the percentage of DL-PCB WHO-TEQ contribution relative to total WHO-TEQs ranges 74.8-83.3% in both species *(Table 1)*. On average, the contribution of mono-*ortho* DL-PCBs is higher than the one by the non-*ortho* DL-PCBs *(Table 1)*, with a predominance of the pentachloro substituted congener 118. A similar contribution range was observed in a German study in fish (eel, chub, ide and bream) collected from the River Elbe and its tributaries [18].

CONCLUSIONS

In the three urban sectors of the river Tiber investigated, some contamination differences were found in the fish sampled. These differences appear to be correlated only in part to the sector sampled. The eel specimen data seem to witness that the Trastevere sector, except for total PCBs, is the most contaminated site whereas the chub data, on the whole, are more erratic. As a general interpretation, the divergence in the results of the different chemicals *(Tables 1)* may be due to the different moving tendencies of the fish. This appears to be confirmed by the inter-site consistency of PCDD and PCDF profiles in the chub *(Figure 1)*. For these chemicals, the eel specimen profiles appear relatively differentiated. Another consideration regards the possible use of these two fish species as bioaccumulation bioindicators. In this respect, the eel appears to be more suitable than the chub to monitor the contaminant of interest. A pronounced site-specific variability in NDL-PCBs and PCDDs + PCDFs data among eel specimens with respect to other fish species has been observed in the cited studies [10, 18]. The use of eel as a bioindicator is currently adopted in Belgium on a routine scale [6]. Anyway, the pronounced species-specific differences between the eel and the chub specimen profiles appear to limit the diagnostic power of these organisms as a source-identification tool. In addition, the eel body burden confirms that these chemicals can have an influence on the worldwide decline of the eel [19]: indeed this species was not included among the fish considered for the chemical monitoring under the Water Framework Directive. For what concerns the Regulation 1881/2006/EC and Recommendation 88/2006/EC, all eel specimens are contaminated above the pertinent maximum levels (ML) of 12 pgWHO-TE/g *ww* and all DL-PCB concentrations found in the chub specimens are above the pertinent action level (AL). Therefore, the situation appears particularly critical because the eel specimens considered in this study have significantly smaller sizes (average weight, 142 g) than the specimens for human consumption, which are marketed when they have a larger size and are eaten by some groups of people.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organization that could inappropriately bias conduct and findings of this study.

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