IDENTIFICATION OF UNKNOWN ORGANOHALOGENATED COMPOUNDS IN EEL SAMPLES FROM BURRISHOOLE CATCHMENT, IRELAND

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Introduction

European eel (*Anguilla anguilla* L.) has been frequently used as a bio-indicator for the monitoring of POPs, because this stage is characterised by primarily sedentary behaviour[1]. In this way, the analysis of eel samples gives a representative description of contamination patterns in the close vicinity of the areas where it was caught. Furthermore, eel is a fish with high body fat percentage and a long life span, assuring an optimal accumulation of lipophilic contaminants, such as PCBs or brominated flame retardants (BFRs)[2,3]. Recently, it has been suggested that PCBs, dioxin-like chemicals and other POPs may play an important role in the actual decline of the European eel[4-6], although causative relationships between chemical exposure and effects on population level are difficult to demonstrate, considering the complex life cycle of this species.

During the Eeliad project (an EU FP7 research project, www.eeliad.com), eels were sampled from 10 different European catchments. This Eeliad project aims through a large-scale field program to determine the migration routes and behaviour of eels during their spawning migration, and to determine ecological factors that influence the number and quality (e.g. lipids, metals and POPs) of silver eels leaving river catchments to start their reproductive migration. Samples have been analyzed for a broad range of POPs, which included PCBs, PBDEs, HBCDs and OCPs. The results of POPs are not presented here.

However, during the analysis of eel extracts from the Burrishoole catchment, western Ireland, a number of peaks with a signal much more intense than that of PBDEs were seen in the ECNI chromatogram, acquired in SIM for *m/z* 79 and 81. It was clear that these unknown compounds were brominated, but their identity could not be confirmed with this analytical setup. We present here the tentative structural identification of these unknown peaks in eels from Burrishoole, Ireland using a number of GC-MS techniques.

Materials and methods

Samples. On December 2008, 13 female eels were caught during their migration along the Burrishoole system (Co. Mayo, western Ireland), which is an oligotrophic and poorly buffered system with an area of 8,949 ha and consists of three main lakes, the brackish lake Furnace (141 ha), the freshwater lakes Feeagh (410 ha) and Bunaveela (46 ha) and a number of smaller lakes. The catchment area is drained by ~ 70 km of small shallow streams, which make up 28 ha of shallow stream area[7]. The area is characterized by absence of industrial activity and by a very low population density and the main land uses are hill sheep farming and commercial coniferous forestry plantations (23% land cover). Due to its volcanic or metamorphic geology, poor buffering capacity and low nutrient content, the lakes in the catchment are relatively acidic, have a low primary productivity which translates to low algal production and slow fish growth. The catchment was never commercially fished for eel. The eels were captured in Wolf-type fish traps situated on two short outflow rivers joining Lake Feeagh to Lake Furnace. Their total length and weight averaged 519±51 mm and 257±67 g. At the lab, fish were measured, weighed, skinned and samples of muscle tissue were removed, labelled and stored at −20°C.

Materials. All solvents were of pesticide grade. Bond Elut-Si was purchased from Agilent and empty polypropylene filtration tubes (3 mL) SPE cartridges were from Supelco. Sodium sulphate and silica gel (0.063-0.200 mm, Merck) were pre-washed and heated overnight at 150°C before use. Empty polypropylene columns for clean-up (25 mL) were purchased from Alltech.

Methods. The sample preparation method used was based on the method described by Roosens et al.[3]. About 1 g pooled eel muscle was weighed, homogenised with Na_2SO_4, spiked with internal standards (CB 143, BDE 77, BDE 128, ^{13}C-α-, ^{13}C-β- and ^{13}C-γ-HBCD) and extracted by hot Soxhlet with hexane:acetone (3:1). Prior to

the clean-up, a portion of the extract was taken to determine gravimetrically the lipid content. The remaining extract was cleaned-up on 8 g acidified silica (44%) and the analytes were eluted with 20 mL hexane and 15 mL DCM. The cleaned extract was evaporated to dryness, redissolved in 0.5 mL hexane and fractionated on pre-packed silica cartridges. A first fraction (A) was eluted with 8 mL hexane and contained PCBs and PBDEs, while the second fraction (B) eluted with 8 ml DCM contained HBCDs. Both fractions were evaporated to incipient dryness and redissolved in 100 µL *iso*-octane (Fr A) and 100 µL methanol (Fr B), respectively.

The fractions were injected on a GC-MS system (Agilent 6890-5973 GC-MS) operated in electron capture negative ionization (ECNI) mode. The GC system was equipped with a PTV inlet which was run in the pulsed splitless mode. One µL of extract was injected on a DB-5 column (15 m × 0.25 mm × 0.10 µm). Methane was used as moderating gas and helium was used as a carrier gas with an initial flow of 1 mL/min (for 20 min) which was then ramped to 2 mL/min. The ion source, quadrupole and interface temperatures were set at 250, 150 and 300°C, respectively and the electron multiplier voltage was at 2200 V. Data acquisition was performed in full scan (range 35 to 800 amu).

Each fraction was injected in an identical GC-MS system operated in the electron ionisation (EI) mode. One µL of extract was injected in pulsed splitless mode on a SGE-HT8 column (25 m × 0.22 mm × 0.25 µm). Helium was used as a carrier gas with a constant flow (1 mL/min). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300°C, respectively and the electron multiplier voltage was at 2200 V. Data acquisition was performed in full scan (range 100 to 800 amu).

A few extracts were analysed on a micrOTOF-MS (Bruker, Daltonik) coupled to a GC (Agilent 7890A) for accurate mass determination. The interface was using atmospheric pressure chemical ionization (APCI) in both negative and positive mode. One µL of extract was injected on a DB-5 column (15 m × 0.22 mm × 0.25 µm). Helium was used as a carrier gas with a constant flow (1 mL/min). The interface temperature was 300°C and data acquisition was performed in full scan (range 100 to 1000 amu). Only negative mode gave results with the settings; capillary +3500 V, Corona -9000V. The dry gas temperature was set at 200°C, the vaporizer temperature at 300°C and the dry gas flow at 2 L/min.

Results and discussion

During the routine analysis of PBDEs on the GC-ECNI/MS using SIM acquisition for ions m/z 79 and 81, a number of unknown peaks have been observed in the region situated between the elution times of BDE 153 and BDE 183. These peaks have been seen only in the eel samples from Burrishoole, Ireland. From our experience, there were no other major PBDEs expected to elute in this region and the profile of the peaks was unique. It was decided to run these extracts in full scan in an attempt to identify them (Figure 1).

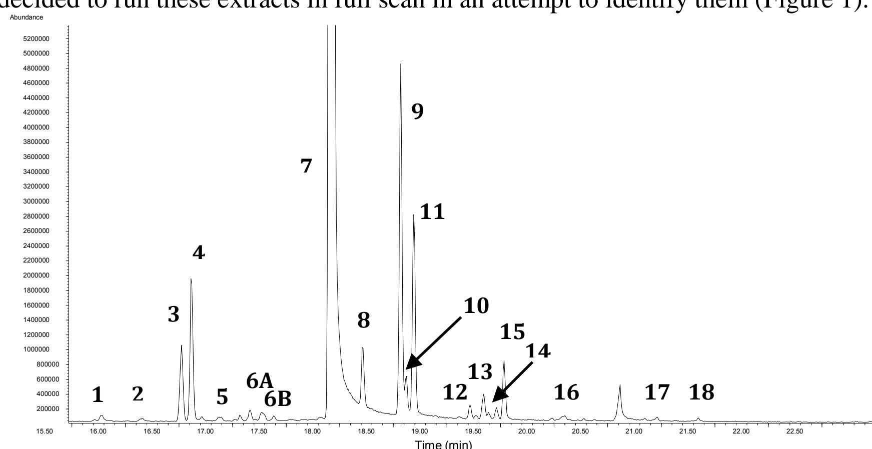

Figure 1. Full scan chromatogram on GC-ECNI MS of a typical muscle eel extract.

Peak 15 (full scan mass spectrum in Figure 2) was identified as octachlorodibenzodioxin (OCDD). McHugh et al.[8] reported that eel samples from Burrishoole were found to contain not only OCDD, but also hepta-CDDs and hexa-CDDs at concentrations almost 100 times higher than other Irish locations. However, PCDFs and PCBs were not different between eel samples from Burrishoole and other locations, indicating a contamination source

specific for that location and which was also specific for OCDD. The identification of OCDD in full scan ECNI-MS is anyway spectacular.

Surprisingly, the total ion chromatogram revealed a major peak (number 7), next to the unknown peaks observed during the SIM acquisition of Br^- ions. Moreover, other peaks were present in the scan chromatogram, yet these peaks do not contain bromine since they gave no signal in the SIM chromatogram (*m/z* 79 and 81)

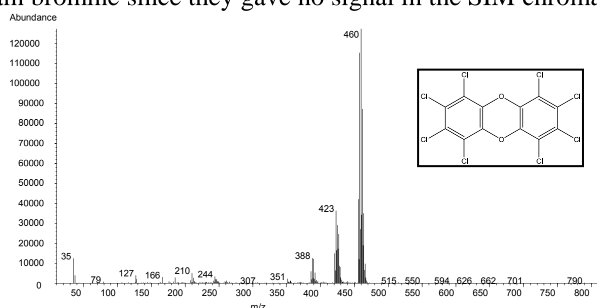

Figure 2. Mass spectrum of compound number 15, tentatively identified as OCDD.

A number of common ions were observed for the compounds detected in Figure 1. Most probably, these compounds have similar structures, thus are isomers, and differ in the number and position of halogens on the rings. For instance, the ion cluster at *m/z* 261 present in compounds 3, 4, 7, 9, 10, 11 is visible only in ECNI-MS which indicates that this negative ion is stable. According to the chlorinated pattern cluster and to the fragment ions observed in the mass spectrum, we believe that this ion cluster corresponds to a phenoxy ion with 4 chlorine and with one methoxy group substituent. Therefore, the structure for compound 7, the major compound, is given here below and corresponds to an octachlorinated diphenyl ether (DE), substituted with 2 MeO- groups (Figure 3). The molecular formula is $C_{14}H_6O_3Cl_8$ and the molecular mass is 506 (monoisotopic mass = 502).

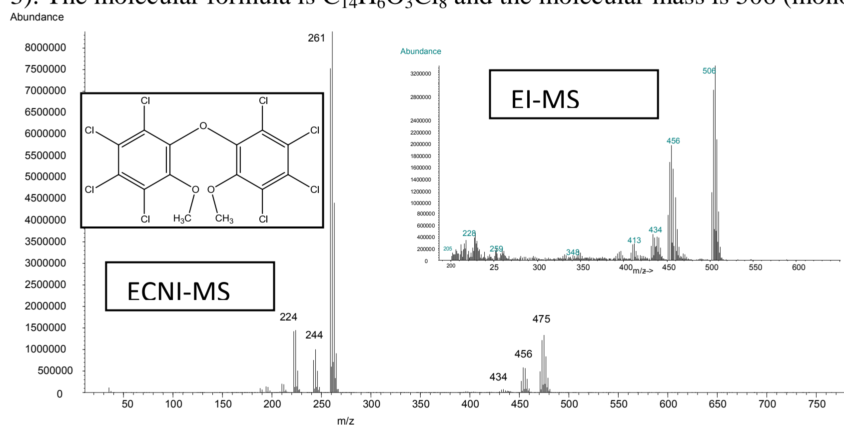

Figure 3. Mass spectrum of compound 7, the major compound found in Irish eel samples from Burrishoole. Interestingly, the molecular ion is absent in the mass spectrum, while ions $[M\text{-}OCH_3]^-$ and $[M\text{-}Cl\text{-}CH_3]^-$ are present.

This structure has been confirmed by acquiring the EI-MS mass spectrum, in which the molecular ion is the base peak, followed by the $[M\text{-}Cl\text{-}CH_3]^+$ ion cluster (Figure 3 - insert). Further, the molecular formula was confirmed by accurate mass determinations by GC-APCI-TOFMS. No mother ion could be detected. The two major fragment ions were $[M\text{-}OCH_3]^-$ and $[C_7H_3O_2Cl_4]^-$. Other typical ions formed (and detected) in APCI negative mode are with an addition of the oxygen radical formed in the APCI source, i.e. $[M\text{-}Cl\text{+}O]^-$ and $[M\text{-}CH_3\text{+}O]^-$. It was not yet possible to perform an internal calibration with the GC-TOFMS, which resulted in a mass deviation of ca 0.02 *m/z* (~50 ppm). This deviation was within the range of the standards injected (OH-PCBs and OH-PBDEs). The accurate mass will be determined with internal calibration.

Several other specific ion clusters have been identified in the various peaks in ECNI-MS. For instance, the ion cluster at *m/z* 265 was once more visible only in ECNI-MS, indicating that similar to ion cluster *m/z* 261, this ion is negative and stable. We believe that this corresponds to a phenoxy ring with five chlorine atoms. The chlorination pattern also confirms our hypothesis. This pattern is present in compounds 1, 5 and 8. We believe

that the possible structures in this case are: heptachlorinated DE (compound 1), octachlorinated DE (compound 5) and nonachlorinated DE (compound 8). Figure 4 gives a representative mass spectrum for this class of compounds, namely polychlorinated diphenyl ethers (PCDEs).

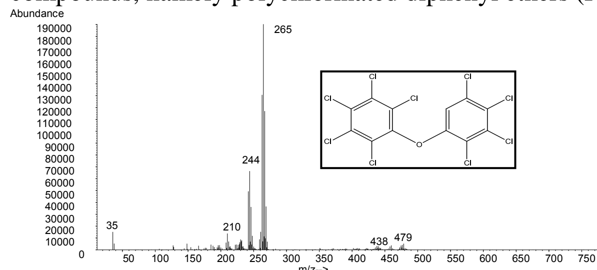

Figure 4. Mass spectrum of compound 8, tentatively identified as nonachlorinated DE. The ion cluster at *m/z* 265 is the base ion and corresponds to a pentachlorophenoxy negative ion. In this case also, the molecular ion is absent in the mass spectrum.

Other interesting ion clusters are those observed at *m/z* 305 (corresponding to a phenoxy ion substituted with 3 chlorine atoms, one bromine atom and one methoxy group) (Figure 5) and at *m/z* 350 (corresponding to a phenoxy ion substituted with 2 chlorine atoms, 2 bromine atoms and one methoxy group). Both these clusters are present only in ECNI, which indicates that only negative ions can stabilize for these structures. The cluster at *m/z* 305 was observed in compounds 6A, 6B, 9, 10, and 11, while the cluster at m/z 350 was observed in compounds 12-14. The $[M\text{-}Br\text{+}O]^-$ corresponding to the suggested chemical formula was observed by GC-APCI-TOFMS, confirming the presence of the Bromine. The major ions were $[M\text{-}CH_3]^-$, $[M\text{-}Cl\text{+}O]^-$ and less intensive were $[C_7H_3O_2Cl_3Br]^-$ and $[C_7H_3O_2Cl_4]^-$. These compounds were the initial “unknown” peaks observed during routine analysis of PBDEs by ECNI-MS.

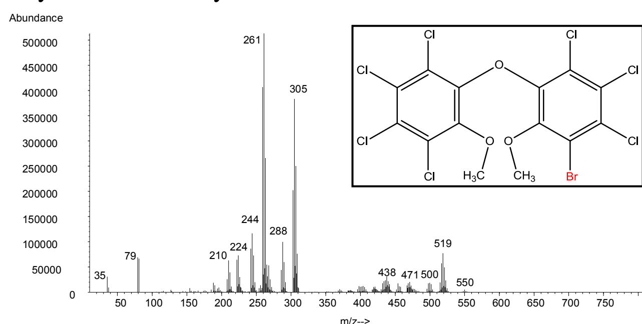

Figure 5. Mass spectrum of compounds 9-11, tentatively identified as heptachlorinated monobrominated dimethoxy DEs. The ion cluster at m/z 261 indicates a fully chlorinated phenoxy ion with 4 chlorine atoms and a methoxy group, while the ion cluster at *m/z* indicated a phenoxy ion substituted with one bromine, 3 chlorine and one methoxy group. The molecular ion is absent or very low in abundance in the mass spectrum.

Elevated OCDD levels in biota have often been associated with pentachlorphenol (PCP) contamination. While PCP levels may be historic in origin, it is not uncommon for the parent PCP to be at low concentrations in biota, with the more persistent by-products of the manufacture of PCP (e.g. OCDD and PCDEs) being accumulated within biota. Analysis of PCP and its more stable metabolic product, pentachloroanisol (PCA), in sediment and eels collected between 2005 and 2009 from the Burrishoole catchment has not shown elevated levels relative to reference locations. Yet, this is not unexpected given the ease of PCP metabolism in organisms. Literature-derived PCDD/F profiles in technical PCP suggest that the PCDD/F profile in Burrishoole eels is more similar to that of PCP formulations rather than to that of other Irish eels.

Acknowledgements: The PhD scholarship of ACI is funded through the Marie Curie ITN INFLAME. GM thanks the Univ. Antwerp for a post-doc fellowship. The authors thank the Eeliad FP7 group for the use of eels.

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