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Accumulation of persistent organic pollutants in consumers of eel from polluted rivers compared to marketable eel☆

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ABSTRACT

Globally, many river sediments are seriously contaminated with persistent organic pollutants (POPs) known to accumulate in aquatic food. In the Netherlands, toxicological risks of human exposure to dioxins and dioxin-like compounds led to a ban on eel fishing in the Rhine-Meuse delta. The aim of this study is to investigate differences in serum POP levels in consumers of eel from high-polluted areas and consumers of eel from low-polluted areas or aquaculture. In total 80 Dutch men were included, aged 40–70 years, with a habitual eel consumption of at least one portion (150 g) per month. Total levels of dioxins and dioxin-like compounds were measured in serum of all participants with the DR CALUX bioassay, validated with GC-MS. For a subgroup of 38 participants extensive POP measurements were performed. We revealed that consumption of eel from polluted rivers resulted in 2.5 and up to 10 times increased levels of dioxins and polychlorinated biphenyls (PCBs) respectively compared to controls. The highest PCB levels were detected for PCB 153, with a median level of 896 ng/g lipid and a maximum level of 5000 ng/g lipid in the high-exposed group. Furthermore, hydroxylated PCB metabolites (OH-PCBs: sum of 4-OH-CB107, 4-OH-CB146, 4′-OH-CB172, and 4-OH-CB187) were 8 times higher in men who consumed eel from polluted areas, and detected at levels (median 4.5 ng/g ww) reported to cause adverse health effects. Also, the majority of the perfluoroalkyl substances (PFASs) were significantly higher in consumers of eel from pullulated areas. In conclusion, this study is the first to reveal that (past) consumption of eel from polluted rivers resulted in high body burdens of dioxins, PCBs, OH-PCBs and PFASs. We confirmed the predictions made in a former risk assessment, and the high levels of dioxins and dioxin-like compounds as well as the OH-PCBs are of health concern.

1. Introduction

Persistent organic pollutants (POPs) are a group of mainly lipophilic compounds that are resistant to degradation and accumulate in the environment and the food chain. POPs include dioxins, polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, brominated flame retardants, and perfluoroalkyl substances (PFASs). These chemicals are known for their potency to cause various adverse health effects, including endocrine disorders, cancer, and neurodevelopmental problems (Li et al., 2006). Many POPs are regulated by the Stockholm Convention, and POP levels in the environment have been decreasing over the past decades (Muir and de Wit, 2010). However, their occurrence in the environment is still a major concern. A high degree of urbanization and industrialization along European rivers have caused the sediments of the main Dutch rivers and estuaries to be highly polluted (den Besten

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et al., 1995). Over 90% of human exposure to POPs comes from food consumption (Liem et al., 2000), with high contributions from fish in general (Bilau et al., 2008; De Mul et al., 2008). Certain fish species dwelling in contaminated areas may contribute even more. In particular eel has potential for high accumulation of contaminants due to eco-physiological features such as bottom dwelling, being a long-living predator, and having a high lipid content (de Boer et al., 2010; Guhl et al., 2014; Kwadijk et al., 2010).

Dioxins (polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs)) and dioxin-like (DL-) PCBs in food have received considerable attention from the European Commission. PCDD/Fs are highly toxic, although with different toxic potencies, as expressed in a range of toxic equivalency factors (TEFs) (Van den Berg et al., 2006). DL-PCBs, including non-ortho (NO) and mono-ortho (MO) PCBs, have properties similar to PCDD/Fs and therefore also assigned TEF values. The NO-PCBs have a higher dioxin-like potency than the MO-PCBs (Van den Berg et al., 2006). To reduce levels in food and hence human exposure, maximum levels (MLs) for PCDD/Fs and DL-PCBs in various food items have been established using toxic equivalents (TEQs) to sum up the different TEF values. In 2012, the ML for the sum of PCDD/Fs and DL-PCBs in wild eel was revised to 10 pg TEQ/g wet weight (ww), while farmed eel and other seafood items have to comply to 6.5 pg TEQ/g ww (EC, 2011). Several studies showed that the majority of eel from the Rhine-Meuse delta exceeded the ML (Guhl et al., 2014; van Leeuwen et al., 2007). Following this discovery, a risk assessment was performed, including the potential impact on the body burden. Long-term consumption of one portion eel (150 g) per month from these high-polluted areas (average level of 29 pg TEQ/g eel) was estimated to result in a body burden of 7.6 ng TEQ/kg body weight (bw), as compared to 3.0 ng TEQ/kg bw for fish eaters not consuming eel (Hoogenboom et al., 2007). Even such moderate eel consumers were therefore expected to reach POP levels above the safe body burden of 4 ng TEQ/kg bw (extrapolated from rat studies), implying that adverse health effects could not be excluded (EU-SCF, 2001; Malarvannan et al., 2014). After a documentary was broadcasted raising awareness, the Dutch government decided on a complete ban on eel fishing from 2011 onwards in the seriously polluted fishing areas, where the majority of the eel exceeded the European maximal levels. Accumulated POPs are very persistent and it takes years before body burdens decrease (half-lives approximately 10–15 years for PCBs; Ritter et al., 2011). Therefore, it is expected that people who consumed this eel regularly in the past will have elevated levels of PCDD/Fs, PCBs and other POPs in their body years after the ban. As the necessity of the prohibited eel fishing is debated, we decided to study the actual body burdens in eel consumers to verify the expectations expressed in the former risk assessment. The aim of this study was to compare blood POP levels in men who consumed eel from high-polluted areas (now closed for eel fishing) with men consuming eel from aquaculture and relatively low-polluted areas (areas open for eel fisheries).

2. Methods

2.1. Study population

The overall setup of the study is depicted in Fig. 1. Eligible participants were all Dutch men aged from 40 to 70, with a long-term habitual eel consumption (in their adult life) of at least one portion (150 g) a month (at least until the implemented ban on eel fishing in 2011). Age range was based on Hoogenboom et al. (2007), who showed that body burden increases due to eel consumption, but remains relatively stable from 40 to 70 years of age. Men were invited to participate through professional and recreational fishermen associations, because these men are more likely to know the origin of the eel, and through advertisements in local newspapers and webpages. A total of 80 men were included, all between February and June 2015, after checking whether they met the inclusion criteria. This study was approved by the Medical Ethical Committee of Wageningen University, and written informed consent was obtained from all participants before inclusion in the study.

Participants filled out a questionnaire at home about their fish consumption habits, including the origin of the eel. The eel was considered to be low-polluted when it came from either aquaculture or areas still open for eel fishery where the eel complies with the European maximum level. The eel was considered to be high-polluted when it came from areas where there is a current ban on eel fishing, as the majority of the eel caught here does not comply with the European maximum level (Rijksoverheid, 2011).

Height and weight of participants were measured in light clothes using standard methods. Blood samples were obtained by venipuncture after overnight fasting, and serum for the persistent organic pollutant (POP) analyses was separated by centrifugation within 6 h after collection. Samples were stored at −80 °C until further analyses. Total cholesterol and triglycerides levels were measured in plasma in a clinical chemistry laboratory (Hospital Gelderse Vallei, Ede, The Netherlands).

2.2. DR CALUX bioassay

Total levels of dioxins and dioxin-like compounds were measured for all 80 subjects using the DR CALUX bioassay at RIKILT Wageningen UR, which was validated for food and feed, as described previously (Bovee et al., 1998). In short, aliquots of 5 mL serum were extracted twice with hexane and purified on a column containing 10 g acid silica (33% H_2SO_4) with 1 g dried Na_2SO_4 on top. The extracts were dried in a SpeedVac with 10 μL DMSO as a keeper, which was later mixed with cell culture medium. Control samples of butter fat with different levels of a mix of PCDD/Fs and DL-PCBs (0.5; 17; 39; and 88 pg TEQ/g lipid), and with a similar absolute amount of fat, were included and extracted in the same way. p-GudLuc transfected H4IIE-cells were obtained from Wageningen University (Murk et al., 1997) and are similar to those sold by Biodetection Systems (BDS, Amsterdam, The Netherlands). Cells were cultured in a 96-wells plate and exposed to the extract (1% DMSO) in quadruplicate for 24 h. The cells were lysed and the luciferase content was measured in a Luminoskan (Labsystems). Total levels of dioxins and dioxin-like compounds were estimated from a calibration curve of the reference butter fat samples included in each clean-up series. These estimated levels were expressed in bioanalytical equivalents (BEQs) to acknowledge the fact that they were determined with a bioassay and not with gas chromatography high resolution mass spectrometry (GC-HRMS). In the bioassay also other compounds that pass the selective clean-up may contribute to the response. BEQ levels were adjusted for lipid content, calculated based on triglycerides and total cholesterol levels as described by Phillips et al. 1989 and recommended by AMAP 2009, which correlates well to gravimetric determination (Bergonzi et al., 2009; Bernert et al., 2007).

2.3. Validation bioassay with GC-HRMS and congener patterns

Eight pooled samples as well as two individual samples with the highest estimated BEQ levels (>100 pg BEQ/g lipid) were measured with GC-HRMS at RIKILT Wageningen UR, which is ISO/IEC 17025 accredited for the analysis of PCDD/F and PCB containing extracts (L014). GC-HRMS was first of all performed to validate the bioassay results, and secondly to compare congener patterns. Pooled serum included samples from all low- and high-exposed men (n = 34 and