Chlorinated paraffins (CPs) in salmon and trout: Occurrence levels, homologue patterns and relation to other persistent organic pollutants

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Introduction

Chlorinated paraffins (CPs) are complex mixtures of various constitutional and optical isomers that are commonly divided by their carbon chain lengths and/or degree of chlorination.[1] CPs are widely used as plasticisers, flame retardants and metal working fluids.[2] Phase-out of short-chain chlorinated paraffins (C_{10}-C_{13}, SCCPs) in Europe, the U.S. and Canada and their classification of POPs by the Stockholm Convention,[3] has prompted an increase in medium-chain chlorinated paraffin (C_{14}-C_{17}, MCCP) production. In 2012, the total CP production was estimated at >1.1 million tonnes/year, with an expected further increase in recent years.[2] In parallel to their intense use, high amounts of these highly persistent compounds have been unintentionally released into the environment.[4] Spread throughout the food web, CPs can ultimately be found as part of the human dietary intake.[5] Therefore, several studies ranging from market-basket studies[6] over examinations of the aquatic food web[7] to area-based studies of wildlife, most recently Asia,[8–11] have been carried out. In this study, SCCPs and MCCP concentrations and homologue patterns were determined in more than 140 salmon and trout samples from the German market by means of GC-Orbitrap-HRMS.[12] In addition, CP concentrations were compared with those of other persistent organic pollutants such as polychlorinated biphenyls (PCBs).

Materials and methods

Salmon (*Salmo salar*), wild salmon and trout (*Oncorhynchus spp.*) samples were acquired from supermarkets and vendors in Baden-Wuerttemberg (Southern Germany) as part of the official food control programme 2014-2017 and prepared as described elsewhere.[12] GC/MS analyses were conducted using a Q Exactive GC Orbitrap mass spectrometer coupled with a TRACE 1310 GC equipped with a TriPlus RSH autosampler (Thermo Scientific, Waltham, MA, USA). A 15 m x 0.25 mm I.D. x 0.25 µm film thickness HP-5MS UI capillary column (Agilent Technologies, Santa Clara, CA, USA) was used with the following short oven temperature program: After 2 min at 60 °C at 50 °C/min to 300 °C which was held for 10 min. Helium Alphagaz 1 (Air Liquide, Düsseldorf, Germany) was used as the carrier gas at a flow rate of 1.4 mL/min. Splitless injections started at 70 °C with a splitless time of 1.2 min, followed by a transfer rate of 12.0 °C/s up until 300 °C. The MS was operated in electron-capture negative ion mode (ECNI) using full scan mode (*m/z* 250-810) at 120,000 mass resolution (FWHM, measured at *m/z* 200). Methane 4.5 (Messer Griesheim, Krefeld, Germany) was used as reagent gas, with gas flow and further specifications of the GC-Orbitrap system described elsewhere.[12] Data processing was done using the Thermo Scientific TraceFinder software. Chain-length specific SCCP and MCCP standards were prepared at the University

of Hohenheim, Institute of Food Chemistry, Stuttgart, Germany with average chlorination degrees between 51 and 61%. Solutions with total concentrations of 0.1, 0.5, 1, 5, 10 and 15 ng/µL were used for calibration after determination of the average response factors according to Yuan *et al*.[13]

Results and discussion

Trout (*Oncorhynchus spp.*) mainly from Baden-Wuerttemberg (n=44) were dominated by high amounts of C_{14}-MCCPs which were surpassing the concentrations of C_{10}- and C_{13}-homologues (Table 1). Generally, a shift towards higher chlorinated homologues was observed, leading to main homologues being $C_{10\text{-}16}Cl_{9\text{-}10}$. Slight differences between the different subgroups (Figure 1, left panel) mainly originated from shifts in the profile of C_{12} and C_{14} homologues with the broad pattern being preserved. This is consistent with the very similar aquatic environment and close relation of the different trout species to each other. Fish sold as "salmon trout" are often rainbow trout fed with astaxanthin to achieve a pink hue of the flesh. Therefore, differences in homologue patterns compared to the regular rainbow trout could be due to the use of different feed.

Table 1. Number of samples within five concentration ranges of SCCPs, MCCPs and total CPs [µg/g fat]. Trout and salmon samples were further divided into their most occurring subgroups.

	SCCPs [ng/g fat]					MCCPs [ng/g fat]					total CP [ng/g fat]					Total no. of samples
	0-100	100-500	500-1000	1000-2000	>2000	0-100	100-500	500-1000	1000-2000	>2000	0-100	100-500	500-1000	1000-2000	>2000	
Trout (n = 44)	1	14	21	8	0	2	21	14	6	1	1	3	16	18	6	44
- rainbow trout	0	5	7	2	0	0	8	2	4	0	0	2	4	6	2	14
- salmon trout	0	4	2	0	0	0	3	3	0	0	0	0	4	2	0	6
- common trout	1	5	12	6	0	2	10	9	2	1	1	1	8	10	4	24
Salmon (n =101)	6	45	35	13	2	8	71	15	4	3	4	21	41	26	9	101
- Norwegian salmon	2	19	12	5	0	4	26	8	0	0	2	9	18	9	0	38
- Scottish salmon	0	3	3	1	0	1	5	0	1	0	0	2	2	3	0	7
- wild salmon	2	5	2	2	2	2	5	1	3	2	2	2	1	3	5	13
- other	2	18	18	5	0	1	35	6	0	1	0	8	20	11	4	43

In a similar way, 101 salmon (*Salmo spp., Oncorhynchus spp.*) samples were analysed. In brief, salmons from aquaculture in Norway and Scotland showed similar CP patterns (with a slightly higher abundance of C_{11} homologues and a shift in the C_{14} profile in Scottish specimen). However, CP patterns in samples labelled "wild salmon" were markedly different (Figure 1, right panel). Differences between arithmetic mean and median (Figure 1, right panel) indicated the presence of several different sub-patterns within this group. This was partly expected as "wild salmon" is a general term used for both Pacific salmon (*Oncorhynchus spp.*) and Atlantic salmon (*Salmo salar*), when caught at sea. The CP homologue pattern (Figure 1, right panel) also suggested a much higher abundance of MCCPs in wild catch than in farmed salmon, hinting at increasing MCCP contamination in the oceans.

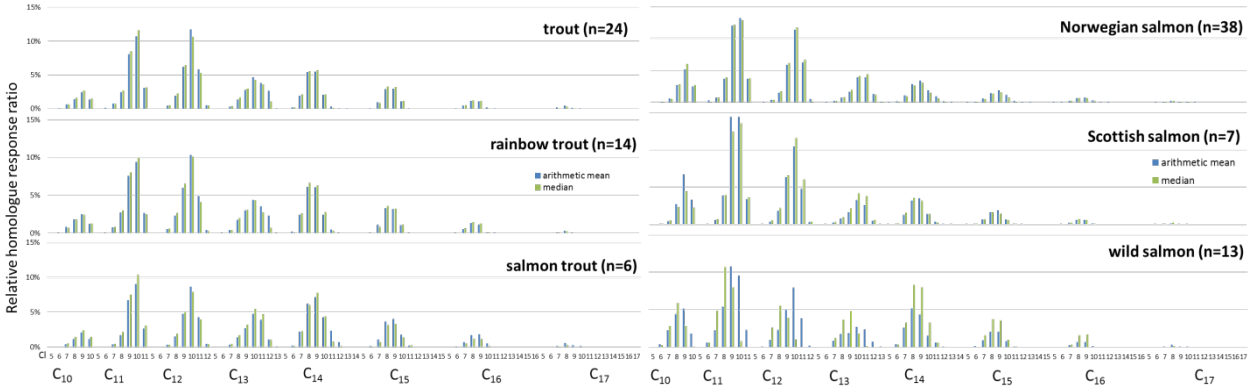

Figure 1. Homologue pattern shown as relative response ratio to the internal standard of 44 trout, divided into rainbow trout, salmon trout and other (unspecified) trout according to food labelling (left) and 58 salmon samples, divided into Norwegian, Scottish and "wild" salmon according to food labelling (right).

Temporal trend for salmonids (not differentiating between the previously mentioned subtypes) were inspected by comparing results from samples collected in 2014, 2016, and 2017. Despite uncertainties created by this broader approach to choosing the samples pool, a clear increase of SCCPs, MCCPs and total CPs was observed between 2014 and 2016 (Figure 2). In the light of increasing production volumes for CPs this trend was expected, merely the comparatively small increase of MCCPs was unexpected. By contrast, CP levels did not increase in samples from 2017 and even were on average slightly lower concentrated in all the CP categories than in 2016. One possible explanation for this could be the recently developing trend to supplement salmon feed with plant based protein instead of fish oil and fish meal, which are believed to be one of the main sources of CPs in fish feed.[14]

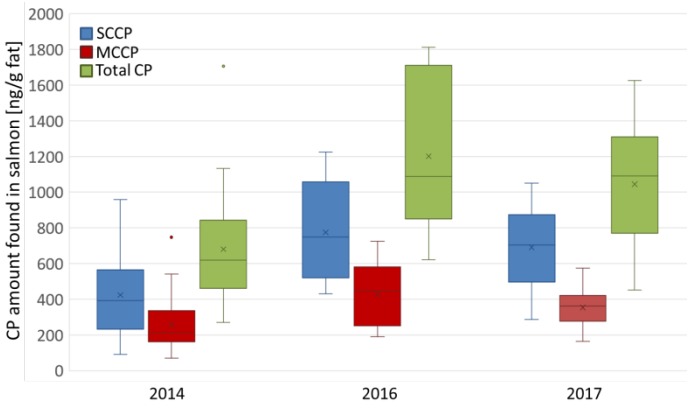

Figure 2. Concentrations [ng/g fat] of SCCPs (blue), MCCPs (red) and total CPs (green) in salmonids between 2014 and 2017 with median (x) and average value (-) given.

Content of CPs and other POPs like PCBs or PDCC/Fs allowed a differentiation between organic farmed salmon (red), wild salmon caught at sea (green) and farmed salmon (blue) (Figure 3). Especially organic samples (orange dots) were rather high in PCDD/F-PCB-TEQ (up to 14 pg TEQ/g fat) while total CPs was comparably low (~500 ng/g fat). Wild salmon samples (green diamond) varied more in their CP content, while TEQs were typically higher than in

conventional farmed salmon (small blue dots). Further investigation into the role of different indicator PCBs or PCDD/Fs is needed to verify these findings.

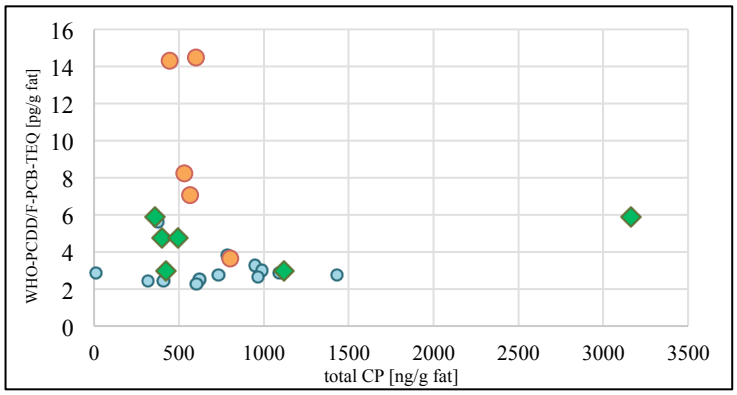

Figure 3. Total CP amount (ng/g fat) of Norwegian farmed (conventional: light blue, organic: orange) and wild salmon samples (green) in comparison to the upper bound of the PCDD/F-PCB-TEQ (in pg/g fat, calculated using the 2005 WHO-TEF).

This study is the first to show MCCP homologue patterns obtained using GC with high resolution, accurate mass Orbitrap-MS technology in >140 fish samples in three recent years (2014, 2016 and 2017). Differences between subgroups of fish could be observed. Comparison of levels of CPs with other POPs may be useful to distinguish samples from different forms of farming in aquaculture.

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