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Two Trace Analytical Methods for Determination of Hydroxylated PCBs and Other Halogenated Phenolic Compounds in Eggs from Norwegian Birds of Prey

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Two new trace analytical methods are presented for identification and quantification of phenolic compounds in complex biological matrixes such as bird of prey eggs. One method is based on derivatization with methyl chloroformate prior to GC/high-resolution MS (HRMS) analysis in electron impact ionization mode. Alternatively, the underivatized phenolic analytes were separated and detected by HPLC coupled to time-of-flight MS (TOF-MS) in the negative ion electrospray ionization mode. For both methods, the egg samples were homogenized and dried with acidified sodium sulfate, cold column-extracted, and cleaned up by gel permeation chromatography and subsequently a Florisil column. Recovery rates for pentachlorophenol (PCP), tetrabromobisphenol A (TBBPA), and selected hydroxylated PCBs (HO-PCBs) from spiked hen's eggs (spiking level 1 ng/g of wet weight (ww)) were in the range of 56−98% for the HPLC/MS method and 57−108% for GC/MS including derivatization. Typical detection limits of the HPLC/TOF-MS method were 5 pg/g ww (1−2 pg injected) for HO-PCBs and PCP and 20 pg/g ww (3 pg injected) for TBBPA. The GC/HRMS method achieved detection limits of ∼1 pg/g ww in predatory bird eggs for all analytes (0.2 pg injected for derivatized TBBPA and 0.05 pg injected for derivatized HO-PCBs and PCP). Eight eggs from four different Norwegian predatory bird species were analyzed. The concentrations determined with the two different quantification methods corresponded well with each other. PCP and TBBPA were found in all samples at concentrations up to 1350 and 13 pg/g ww, respectively (GC/HRMS values). A total of 55 penta- to nonachloro-HO-PCB congeners were detected in the eight eggs, 10 of those could be structurally identified. The maximum HO-PCB congener concentration was found for 4-HO-CB 187 in a peregrine falcon egg with estimated 388 pg/g ww. Another peregrine falcon egg was highest contaminated with sum HO-PCBs (estimated 2.1 ng/g ww). This level was 1.2‰ of the sum PCBs value for the same egg. Furthermore, indications were found that the HO-PCB congener distribution pattern could be species specific for predatory birds.

Polychlorinated biphenyls (PCBs) often dominate the organohalogen contaminant burden in biological samples.[1,2] Considerable knowledge about the distribution and fate of this compound class in the environment was gathered in thorough research work during the last 40 years. PCBs are recognized as persistent, bioaccumulative compounds, able to induce a broad spectrum of toxicological effects, for example, by influencing hormone metabolism.[3−8] Hitherto, however, the levels and effects of the hydroxylated metabolites of PCBs and other phenolic organohalogens in the environment have been scarcely studied.

According to generally accepted scientific knowledge, PCBs are mainly biotransformed by the cytochrome p-450 monooxygenase system and formation of hydroxylated PCBs (HO-PCBs) is the major metabolic pathway. Even the most persistent PCB congeners, such as CB 118, CB 138, and CB 153, are known to be transformed to a number of hydroxylated metabolites.[9−12] Most of the HO-PCB congeners are readily conjugated and excreted. However, a subset of HO-PCBs are strongly retained in humans and wildlife due to their binding to the thyroid hormone transport protein transthyretin.[10,13,14] HO-PCBs have been detected in human plasma,[14−17] gray seal blood,[14] and fish blood plasma,[18,19] as well as bird eggs,[20] and some congeners have been quantified at levels

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comparable to the parent PCBs. In recent years, hydroxylated PCB metabolites were also included in toxicological research[21−23] mainly due to their structural similarities to hormones, for example, 17β-estradiol and thyroxin.

Pentachlorophenol (PCP), a fungicide used as preservation additive to leather, timber, textiles, and paper as well as in cotton farming is another antropogenic substance mimicking thyroxin. In 1991, the annual global production of PCP was estimated to be 25 000−90 000 t and its universal use during decades led to an extensive contamination of the environment. Tetrabromobisphenol A (TBBPA) belongs to the reactive brominated flame-retardants and is a representative of brominated phenolic xenobiotics. In vitro competition experiments showed that TBBPA has a 10 times higher binding affinity to transthyretin than the natural ligand thyroxin.[24]

Owing to their high trophic position, birds of prey are important indicator organisms for antropogenic pollution. The negative influence of chlorinated hydrocarbon contamination on their productivity has been demonstrated in a large number of reports.[6,25,26] Birds of prey feeding on the terrestrial and on the marine food chain are characterized by high pollutant levels.[27] The investigation of addled eggs proved to be a very useful method to characterize the contamination of the mother organism and the offspring.[26,28,29] Studies investigating the fate of orally administered DDT in laying hens proved that both the parent compound DDT and its metabolites were distributed in the main tissues involved in egg formation as well as in the egg yolk.[30] Likewise, hydroxylated PCB metabolites can be transferred to bird eggs from the mother organism prior to egg laying.[20] These findings imply that the mother organism was exposed to HO-PCBs either through intake via feed or by its own ability to metabolize PCBs.

Most hitherto published studies dealing with determination of halogenated phenolic compounds in environmental samples employed the same general analytical strategy.[14−20,31] It consists of extraction of analytes using organic solvents, partitioning from the neutral fraction with aqueous potassium hydroxide, acidification, and re-extraction into an organic phase, derivatization with diazomethane, and a final cleanup step before analysis by gas chromatography (GC)/electron capture negative ionization MS. This general method has been applied for analysis of HO-PCBs,[14−20] PCP,[15,17−19] and TBBPA.[31]

The objective of the present study was to develop and validate complementary, highly sensitive, and reliable analytical methods for the determination of HO-PCBs and other phenolic compounds in biota. These methods should be applicable even to very complex matrixes such as predatory bird eggs. A GC method was developed based on derivatization of the target analytes with methyl chloroformate and detection by electron impact MS. In addition, a complementary separation and detection technique based on high-performance liquid chromatography (HPLC) coupled to time-of-flight mass spectrometry (TOF-MS) of underivatized phenolic organohalogens was developed. To our best knowledge, this is the first published HPLC/MS method dealing with environmental levels of HO-PCBs. Employing two different instrumental techniques is recommended, since many of the HO-PCBs found in biological samples are hitherto not structurally characterized and no relevant reference compounds are commercially available.

EXPERIMENTAL SECTION

Chemicals. In this work, HO-PCBs and their derivatives are designated as described by Letcher et al.,[32] using the well-established PCB nomenclature and numbering the (derivatized) hydroxyl group thereafter. This deviates from IUPAC guidelines, which give the hydroxyl group highest priority, but allows direct structural comparison with PCBs and facilitates comparison with publications by other research groups.[15,17,33,34] The following HO-PCBs (100 μg/mL in isooctane), used for method development and quantification, were purchased from AccuStandard Inc. (New Haven, CT): 4-hydroxy-3,5-dichlorobiphenyl (4-HO-CB 14), 4-hydroxy-2′,3,5′-trichlorobiphenyl (4′-HO-CB 26), 3-hydroxy-2′,3′,5′,6′-tetrachlorobiphenyl (3′-HO-CB 65), 4-hydroxy-2′,3′,5′,6′-tetrachlorobiphenyl (4′-HO-CB 65), 4-hydroxy-2′,3,3′,4′,5′-pentachlorobiphenyl (4′-HO-CB 106), 4-hydroxy-2′,3,4′,5,6′-pentachlorobiphenyl (4′-HO-CB 121), 4-hydroxy-2′,3,3′,4′,5,5′-hexachlorobiphenyl (4′-HO-CB 159), and 4-hydroxy-2′,3,3′,5,5′,6′-hexachlorobiphenyl (4′-HO-CB 165). $^{13}C_{12}$-labeled 4-hydroxy-2,2′,3,4′,5,5′,6-heptachlorobiphenyl ($^{13}C_{12}$-4-HO-CB 187, chemical >98%, isotopic 99%, 50 μg/mL in toluene) was obtained from Wellington Laboratories Inc. (Guelph, ON, Canada), pentachlorophenol (PCP, 98%) from Sigma-Aldrich AS (Oslo, Norway), and $^{13}C_6$-PCP (103 ppm in nonane) from Cambridge Isotope Laboratories (Andover, MA). Tetrabromo-

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Table 1. Analyzed Eggs from Norwegian Birds of Prey[a]

ID	species	scientific name	main diet	year	place	region
O1	osprey[b]	*Pandion haliaetus*	freshwater fish	1994	Svelvik	southeastern coast
O2				2000	Os i Hedmark	central mountains
GE1	golden eagle	*Aquila chrysaetos*	mammals and game birds	1998	Os i Hedmark	central mountains
GE2				2000	Selbu	inner central Norway
SE1	white-tailed sea eagle	*Haliaeetus albicilla*	fish and seabirds	2000	Vikna	western coast, central Norway
SE2				1996	Lødingen	western coast, north of central Norway
P1	peregrine falcon[b]	*Falco peregrinus*	medium-sized birds	1995	Telemark	southeastern coast
P2				2000	Nærøy	western coast, central Norway

[a] The sample identification code (ID) used in this work as well as sampling year, place, and region are given. [b] Migrating birds.

bisphenol A (TBBPA, technical) was provided by LGC Promochem AB (Borås, Sweden) and 1,2,3,4-tetrachloronaphthalene (TCN, 10 μg/mL in isooctane) by Dr. Ehrenstorfer GmbH (Augsburg, Germany). All solvents and reagents used in this work were of best commercially available quality. Gases (5.0 quality) were purchased from Hydrogas (Porsgrunn, Norway).

Qualitative solutions of the following methoxylated PCBs (MeO-PCBs, ~5 μg/mL in isooctane) were obtained from the working group of Prof. Åke Bergman (Department of Environmental Chemistry, Stockholm University, Sweden): 4-MeO-CB 107, 4′-MeO-CB 130, 3′-MeO-CB 138, 4-MeO-CB 146, 3-MeO-CB 153, 4-MeO-CB 162, 4′-MeO-CB 172, 3′-MeO-CB 180, 4-MeO-CB 187, and 4-MeO-CB 193. The methoxylated PCBs were cleaved to their corresponding HO-PCBs according to Press.[35] Briefly, 500 μL of each of the MeO-PCB solutions was evaporated to dryness under a gentle stream of nitrogen. The residues were dissolved in dichloromethane (dried over anhydrous sodium sulfate) and allowed to react with a slight molar excess of boron tribromide (≥99%, Fluka Chemie GmbH, Buchs, Switzerland) at room temperature for 24 h under nitrogen atmosphere. The reaction mixture was treated with 200 μL of water and then added to 1 mL of 2 M potassium hydroxide in ethanol/water (1:1; v/v). The aqueous phase was washed twice with 1 mL of cyclohexane, acidified with 1 mL of 2.5 M hydrochloric acid in water and extracted twice with 1.5 mL of cyclohexane/diethyl ether (2:1; v/v). The two organic extracts were combined, dried over sodium sulfate, and evaporated to dryness to yield the HO-PCB products. These were either dissolved in methanol/water (1:1; v/v) or derivatized as described below for use as identification standards in HPLC/TOF-MS or GC/HRMS, respectively.

Egg Samples. The hen's eggs used for method development and validation were purchased in a local supermarket in Tromsø, Norway. None of these eggs contained detectable amounts of HO-PCBs. Predatory bird eggs analyzed in this study were provided by the Norwegian Institute for Nature Research (Trondheim, Norway). Unfertilized, abandoned eggs were collected up to several weeks after the hatching period. Two eggs of each of four different bird of prey species were chosen for analysis. They are described in detail in Table 1.

Extraction. The whole content of the egg was homogenized using a blender (Ultraturrax T 25, Janke & Kunkel, IKA Labortechnik, Staufen, Germany). Approximately 10 g of the homogenized sample was mixed with 60 g of pretreated (12 h at 600 °C) and subsequently acidified sodium sulfate (1% (w/w) sulfuric acid); the mixture was kept overnight at −18 °C, allowed to warm to room temperature, and transferred into a glass column. $^{13}C_{12}$-4-HO-CB 187, 4′-HO-CB 26, and to three samples (O2, GE2, SE1) additionally $^{13}C_6$-PCP were added as internal standards/surrogate standards (20 ng each: 20 μL of a 1 μg/mL solution in isooctane). The dry homogenate was extracted three times with cyclohexane/acetone (3:1; v/v) by filling 50 mL of the solvent into the column, allowing it to stand for 1 h, and letting the extract slowly run out of the column. The volume of the combined extracts was reduced to ~4 mL in a Turbovap evaporator (Zymark, Hopkinton, MA) while changing the solvent to dichloromethane.

Cleanup. Gel permeation chromatography (GPC) was applied, using a dual prepacked Envirogel system (packing material: Bio-Beads S-X3 resins, 37−75-μm particles; column 1, 150-mm length, 19-mm i.d.; column 2, 300-mm length, 19-mm i.d.; Waters, Milford, MA). The crude extract (1.5 mL) was injected, and lipid removal was performed at a flow rate of 5 mL/min dichloromethane. The fraction between 72.5 and 115 mL containing all compounds of interest was concentrated to 0.5 mL (Turbovap) while changing the solvent to cyclohexane. Phenolic compounds were additionally cleaned up and separated from neutral organohalogens using a Florisil column manually packed with 1.5 g of pretreated (12 h at 450 °C) and deactivated (0.5% (w/w) water) Florisil (particle size: 0.15−0.25 mm) and 2 g of anhydrous granulated sodium sulfate on top. The column was prewashed with 10 mL of *n*-hexane/dichloromethane (3:1; v/v) before the sample, dissolved in 0.5 mL of cyclohexane, was applied. Neutral compounds and remaining matrix were eluted with 11 mL of *n*-hexane/dichloromethane (3:1; v/v) and subsequently 2 mL of *n*-hexane/acetone (85:15; v/v). These fractions were discarded. The phenolic compounds of interest were then eluted with additionally 4 mL of *n*-hexane/acetone (85:15; v/v) and finally 10 mL of dichloromethane/methanol (88:12; v/v). These last two fractions were combined, concentrated to 1 mL (Turbovap), and split into two equal subsamples for HPLC/TOF-MS and derivatization prior to GC/HRMS analysis, respectively.

HPLC/TOF-MS Analysis. Subsamples from the Florisil cleanup were evaporated to dryness under a gentle stream of nitrogen. A 200-μL aliquot of a 50 ng/mL solution of 4-HO-CB 14

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Table 2. Ion Mass-to-Charge Ratios of the Derivatized Analytes and Standards Used as Quantification Masses (QM) and Control Masses (CM) in Selected Ion Monitoring Mode for GC/LRMS and GC/HRMS[a]

	GC/LRMS		GC/HRMS				
analyte	quant mass	control mass	quant mass	control mass	theor isotopic ratio QM/CM	internal std used	recovery std usd
TCN	265.9	263.9	265.9038	263.9067	1.28		
$^{13}C_{12}$-4-MCF-O-CB 187	437.9	435.9	437.8504	435.8533	1.04	TCN	
$^{13}C_6$-MCF-PCP	270.9	272.9	270.8564	272.8534	1.56	TCN	
4′-MCF-O-CB 26	330.0	332.0	329.9617	331.9588	1.02	TCN	
MCF-PCP	264.8	266.8	264.8362	266.8333	1.56	$^{13}C_{12}$-4-HO-CB 187	TCN
$(MCF)_2$-TBBPA	556.8	554.8	556.7608	554.7629	1.47	$^{13}C_{12}$-4-HO-CB 187	TCN
tetrachloro-MCF-O-PCB	365.9	363.9	na[b]	na	1.30	$^{13}C_{12}$-4-HO-CB 187	TCN
pentachloro-MCF-O-PCB	340.9	342.9	340.8675	342.8646	1.55	$^{13}C_{12}$-4-HO-CB 187	TCN
hexachloro-MCF-O-PCB	389.9	391.9	389.8520	391.8491	1.24	$^{13}C_{12}$-4-HO-CB 187	TCN
heptachloro-MCF-O-PCB	na	na	425.8101	423.8131	1.04	$^{13}C_{12}$-4-HO-CB 187	TCN

[a] Theoretical isotopic ratios QM/CM as well as internal and recovery standards used for quantification of each analyte are also given. [b] na, not analyzed.

(recovery standard/volume standard) in methanol/water (1:1; v/v) was added to the residue, and the mixture was placed into an ultrasonic bath for 10 min. The resulting suspension was filtered through a double-folded Kleenex tissue covering the tip of a Pasteur pipet while it was transferred into a HPLC autoinjector vial. A sample volume of 20 μL (solvent methanol/water 1:1; v/v) was injected into the separation system using an autoinjector and a low-pressure quaternary gradient pump with vacuum degasser (1100 Series, Agilent Technologies, Palo Alto, CA). HPLC separation was carried out on a C_{18} reversed-phase column (Ace 3 C18: 3-μm particles, 100-Å pore size, 150-mm length, 2.1-mm i.d., Advanced Chromatography Technologies, Aberdeen, Scotland) applying a flow rate of 200 μL/min of 1 mM ammonium acetate in both methanol (A) and water (B). The following binary gradient was used: 0−1 min, 50% A; 1−12 min, linear change to 99% A; followed by 10-min rinsing with 99% A. To distinguish between HO-PCBs coeluting on the C_{18} column, the samples were also analyzed on a phenyl reversed-phase column (Ace 3 Phenyl: 150-mm length, 2.1-mm i.d., Advanced Chromatography Technologies) applying the same mobile phase and gradient as described before. A time-of-flight mass spectrometer (LCT, Micromass, Manchester, England) was used employing electrospray ionization in the negative ion mode (ESI(−)). Mass spectra were registered in full-scan mode (mass range m/z 230−550). Instrument parameters were optimized to obtain maximum ion current of the $[M - H]^-$ isotope ion cluster of 4′-HO-CB 159. For this purpose, a solution of 4′-HO-CB 159 (10 μg/mL in methanol; syringe pump, 10 μL/min flow) was continuously added via a T-piece to a flow of 190 μL/min methanol/water (7:3) from the HPLC pump. The following optimized instrument parameters were applied: sample cone voltage, −35 V; desolvation temperature, 350 °C; source temperature, 130 °C; nitrogen cone gas flow, 10 L/h; desolvation gas flow, 400 L/h; nebulizer gas flow, maximum (corresponding to ∼90 L/h).

Derivatization. Subsamples from the Florisil cleanup were evaporated to dryness under a gentle stream of nitrogen. A 150-μL aliquot of the derivatization solvent acetonitrile/methanol/water/pyridine (5:2:2:1; v/v/v/v) was added to the residues, and the mixture was placed into an ultrasonic bath for 10 min. The resulting suspension was filtered through a double-folded Kleenex tissue covering the tip of a Pasteur pipet. A 120-μL aliquot of the clear filtrate was transferred into a 1.5-mL glass vial. Alternatively, HO-PCB standard solutions were evaporated to dryness in a 1.5-mL glass vial, and the residues were redissolved in 120 μL of derivatization solvent. A 10-μL aliquot of methyl chloroformate (MCF; >98%; Merck-Schuchardt, Hohenbrunn, Germany) was added, and the reaction mixture was allowed to stand for 5 min before it was diluted with 300 μL of water. The aqueous solution was extracted twice with 500 μL of isooctane, and the combined extracts were dried over sodium sulfate. After addition of 2 ng of TCN (recovery standard/volume standard; 20 μL of a 100 ng/mL solution in isooctane), the solution was transferred to a GC autoinjector vial and the volume was reduced under a stream of nitrogen to ∼30 μL. In this study, the MCF derivatization products of HO-PCBs, PCP, and TBBPA are designated as MCF-O-PCBs, MCF-PCP, and $(MCF)_2$-TBBPA, respectively.

GC/LRMS Analysis. GC/low-resolution mass spectrometry (LRMS) was used for method development and validation. Samples were analyzed on a Mega II 8065 gas chromatograph (Fisons, Milan, Italy) coupled to an MD800 low-resolution quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) operated in the electron impact mode (GC/EI-MS). Sample volumes of 2 μL (solvent isooctane) were injected on-column with an AS800 autoinjection system (Fisons) onto a DB5MS capillary column (phenyl arylene polymer, 30-m length, 0.25-mm i.d., 0.25-μm film thickness, J&W Scientific, Folsom, CA). Separations were performed using helium carrier gas at a constant column head pressure of 80 kPa, applying the following temperature program: 70 °C (hold time 2 min), then at 15 °C/min to 180 °C (hold time 0 min) and at 5 °C/min to the final temperature of 280 °C (hold time 15 min). The GC/MS interface temperature was held at 250 °C, and the EI ion source temperature at 220 °C. The mass spectrometer was operated in single ion monitoring mode (SIM) using the ion m/z values given in Table 2. For each compound, a quantification mass and a control mass were acquired.

GC/HRMS Analysis. GC/high-resolution mass spectrometry (HRMS) was used for analysis and quantification of the bird egg samples. Sample volumes of 1 μL (solvent isooctane) were injected splitless (2 min splitless time) with a Hewlett-Packard 7673 autoinjector, and separation was carried out on a Hewlett-Packard 5890 Series II gas chromatograph using a DB5MS capillary column (30-m length, 0.25-mm i.d., 0.1-μm film thickness, J&W

Scientific). The gas chromatographic conditions were as follows: carrier gas, helium; constant column head pressure, 170 kPa; injector temperature, 265 °C; GC/MS interface temperature, 280 °C. The same temperature program as described for GC/LRMS was used. Quantification was performed using a VG Autospec Ultima mass spectrometer (Micromass) at a resolution of 10 000 (5% valley). The MS was operated in the EI mode (35 eV) with an ion source temperature of 275 °C and an acceleration voltage of 8000 V. Fragments of perfluorokerosene with less than 13% relative mass difference from the analyte ions were used as lock and control masses. Mass spectra were acquired in SIM using the ion m/z values given in Table 2. A quantification mass and a control mass were acquired for each compound.

Recovery Experiments and Quantification. Recovery rates for standard HO-PCBs, PCP, and TBBPA were determined from spiked hen's egg samples at spiking levels of individual compounds of 100 pg/g, 1 ng/g, and 10 ng/g. For both methods, HPLC/TOF-MS and GC/LRMS, recovery experiments were performed in duplicates (100 pg/g and 10 ng/g level) or three replicates (1 ng/g level). Quantification was performed using the internal standard method. $^{13}C_{12}$-4-HO-CB 187 was spiked as internal standard/surrogate standard to all predatory bird egg samples and $^{13}C_6$-PCP to three of them (O2, GE2, SE1). It was shown that recoveries of $^{13}C_6$-PCP and $^{13}C_{12}$-4-HO-CB 187 were comparable (see Results and Discussion, Method Validation and Comparison). Therefore, $^{13}C_{12}$-4-HO-CB 187 was used as internal standard for quantification of all compounds of interest in all samples. Additionally, 4′-HO-CB 26 was spiked to the homogenized egg samples to control the recovery of lower chlorinated HO-PCBs. Integration was carried out in the acquired SIM mass chromatograms for GC/MS (see Table 2) or in extracted mass chromatograms (typical mass tolerance 0.04 u) of the two most abundant ions of the $[M - H]^-$ isotope cluster for HPLC/full-scan TOF-MS analysis. In real samples, analytes were identified by retention time and an abundance ratio of the two analyzed isotope masses that did not deviate more than 15% from the theoretical value (Table 2 for GC/MS). Since quantitative standards of environmentally relevant HO-PCBs were not available, quantification of pentachloro HO-PCBs was based on the averaged relative response factors (rrf) of 4′-HO-CB 106 and 4′-HO-CB 121; for hexachloro HO-PCBs, the average of the rrf of 4′-HO-CB 159 and 4′-HO-CB 165 was used and hepta- to nonachloro congeners were quantified by applying the response factor of $^{13}C_{12}$-4-HO-CB 187 (or the corresponding MCF derivatives in GC analysis). The quantitative results for HO-PCBs must therefore be considered as estimates. 4-HO-CB 14 and TCN were used as recovery standards in HPLC/TOF-MS and GC/MS, respectively.

PCB Analysis. Analysis of PCBs in the bird of prey egg samples was performed as described elsewhere.[27]

RESULTS AND DISCUSSION

Sample Preparation. Spiking experiments might not adequately reflect the matrix interactions of naturally embedded analytes. Therefore, it is difficult to estimate extraction efficiencies from animal tissues without performing feeding studies with isotopically labeled surrogate standards. However, extraction of phenolic compounds from spiked hen's egg samples was evaluated for sample homogenates with neutral and with acidified sodium sulfate (1% (w/w) sulfuric acid). Recoveries for individual HO-PCB congeners (quantified by GC/LRMS) using neutral and acidified sodium sulfate were 38−67 and 56−94%, respectively. The improved extraction efficiency in the latter case is probably due to the shift in equilibrium between protonated and ionic phenolic analytes or due to partial denaturation of proteins by the sulfuric acid. After lipid removal by GPC, a Florisil column was used for separation of the phenols from neutral organohalogens such as PCBs and organochlorine pesticides. Liquid−liquid partitioning with potassium hydroxide as applied by other working groups[17−20,36] was also tested. However, better recovery rates were achieved by employing the Florisil column, which, in addition, provides a further purification of the sample extract. Recoveries of HO-PCBs (standard solution) in the liquid−liquid partitioning step and employing the Florisil column were 52−108 and 71−98%, respectively. Egg sample extracts from the Florisil column were clean enough for direct injection into the HPLC/TOF-MS system after solvent change and a final filtration step (see Experimental Section).

MCF Derivatization of Phenolic Compounds. GC separation demands derivatization of phenolic compounds, which is done in most laboratories with diazomethane resulting in methoxy derivatives.[17−20,31,36] As an alternative technique, derivatization with MCF was applied in this study, based on the following reaction:

$$R{-}OH + Cl{-}CO{-}O{-}CH_3\ (MCF) \rightarrow R{-}O{-}CO{-}O{-}CH_3 + HCl \quad (R = \text{aromatic ring system})$$

There are many advantages of this derivatization technique. Use of diazomethane can be omitted, MCF is stable over a long period of time and easy to use, and the reaction is performed in less than 5 min at room temperature with excellent reproducibility (less than 10% GC/MS area difference of derivatized standard HO-PCBs in three repetitions at three different days after correction with the volume standard, TCN). In addition, the reaction is not sensitive to water and no further cleanup after derivatization is needed. Most importantly, MCF derivatives of phenols show excellent GC/MS properties, i.e., sharp peak shapes and good response in both EI-MS and electron capture negative ionization-MS. Furthermore, the derivatization method can be used to discriminate between naturally occurring TBBPA and Me-TBBPA, both of which remain in the phenolic fraction during sample cleanup and both are derivatized to $(Me)_2$-TBBPA using diazomethane. It is difficult to estimate recoveries of the MCF derivatization, since standards have to be derivatized before GC/MS analysis as well. On the other hand, in real sample quantification, this might correct for incomplete derivatization. However, some underivatized HO-PCBs gave reasonable peak shapes in GC separation (results not shown). Analysis of remaining hydroxylated PCB after derivatization of standard compounds showed that more than 95% of the hydroxyl groups had reacted. Disadvantages of MCF derivatization are the complex composition of the solvent that could influence the reaction yield and the instability of some of the derivatives toward concentrated sulfuric acid treatment, often applied in the cleanup of biological sample matrixes. Derivatization with MCF should therefore be performed with an already cleaned extract as a final step before instrumental analysis.

(36) Hovander, L.; Athanasiadou, M.; Asplund, L.; Jensen, S.; Klasson Wehler, E. *J. Anal. Toxicol.* **2000**, *24*, 696−703.

Table 3. Limits of Detection (LOD, S/N = 3/1) and Quantification (LOQ, S/N ≥ 10/1, and Signal ≥ Five Times Blank Value) for the Instrumental and the Complete Methods as Well as Recoveries and Repeatability (Relative Standard Deviation (RSD), n = 3) for Hen's Eggs Spiked with 1 ng/g of Wet Weight (ww) of Each Phenolic Compound

	tri- to hexachloro-HO-PCB[a]	$^{13}C_{12}$-4-HO-CB 187[a]	pentachloro-phenol[a]	tetrabromo-bisphenol A[a]
HPLC/TOF-MS				
instrumental				
LOD (pg)	2	1	2	3
spiked hen's egg				
LOD (pg/g ww)	5	4	5	20
LOQ (pg/g ww)	20	15	100	50
recovery (%)	56−80	94	98	79
RSD (%)	<10	4	7	8
GC/LRMS				
instrumental				
LOD (pg)	0.1	0.5	0.3	1
spiked hen's egg				
LOD (pg/g ww)	10	20	10	10
LOQ (pg/g ww)	50	100	100	50
recovery (%)	59−79	92	108	57
RSD (%)	<15	2	9	14
GC/HRMS				
instrumental				
LOD (pg)	0.05	0.1	0.05	0.2
bird of prey eggs				
LOD (pg/g ww)	1	3	1	1
LOQ (pg/g ww)	10	15	100	3

[a] Analyzed as the corresponding MCF derivatives in GC analysis.

Table 4. PCP and TBBPA Concentrations Found in the Bird of Prey Eggs Analyzed by HPLC/TOF-MS and GC/HRMS[a]

	HPLC/TOF-MS	GC/HRMS	
	PCP (pg/g ww)	PCP[b] (pg/g ww)	TBBPA[b] (pg/g ww)
blank	19	20	
O1	(1920)[c]	1350	10
O2	<LOQ	<LOQ	<LOQ
GE1	272	267	13
GE2	174	125	<LOQ
SE1	<LOQ	<LOQ	<LOQ
SE2	209	173	7.2
P1	175	110	<LOQ
P2	<LOQ	<LOQ	4.2

[a] Detected analytes below the quantification limit are designated with <LOQ (see Table 3 for LOD and LOQ values). [b] Analyzed as MCF−PCP and $(MCF)_2$-TBBPA, respectively. [c] Outside the linear range of the detector.

Method Validation and Comparison. Instrumental methods using standard solutions and complete methods using spiked hen's eggs as sample surrogates were validated both for HPLC/TOF-MS of phenolic analytes and for GC/LRMS of MCF derivatives. Results of the validation are summarized in Table 3. Due to insufficient sensitivity of the GC/LRMS method used in the beginning of the study, bird of prey eggs were analyzed by GC/HRMS instead. Detection and quantification limits for this method are also given in Table 3. Special attention has to be paid to the restricted linear range of the detector when working with TOF-MS. In this study, the linear range for all analytes applying the

Table 5. Pentachloro to Nonachloro HO-PCBs Detected in the Predatory Bird Eggs by HPLC/TOF-MS[c]

	pentachloro HO-PCB (Cl_5)					hexachloro HO-PCB (Cl_6)							heptachloro HO-PCB (Cl_7)								octachloro HO-PCB (Cl_8)				Cl_9
	a[a]	**b**	**c**	**d**	**e**	**f**	**g**[a]	**h**[a]	**i**[a]	**j**[a]	**k**[a]	**l**	**m**	**n**	**o**	**p**[a]	**q**	**r**	**s**[a]	**t/u**[a]	**v**	**w**	**x**	**y**	**z**
rRt[b]	1.34	1.35	1.39	1.42	1.48	1.28	1.33	1.34	1.37	1.38	1.43	1.44	1.45	1.27	1.29	1.31	1.34	1.35	1.38	1.43	1.28	1.32	1.36	1.39	1.40
O1		24		<LOQ					47	<LOQ			20	<LOQ	<LOQ	54	<LOQ		<LOQ	22	21	<LOQ	<LOQ	17	
O2									<LOQ							<LOQ				<LOQ	<LOQ		<LOQ		
GE1									<LOQ	<LOQ						15			<LOQ	16				<LOQ	
GE2														<LOQ		<LOQ					<LOQ				
SE1		80	v		<LOQ				90	<LOQ	<LOQ		<LOQ	<LOQ	21	86	<LOQ		<LOQ	<LOQ	45	16	<LOQ	46	18
SE2		57	<LOQ		<LOQ				37	27						47			<LOQ	<LOQ	24		<LOQ	<LOQ	
P1	<LOQ	179	<LOQ	<LOQ	96	191	22	25	158	41	<LOQ	<LOQ	<LOQ	101	39	363	26	16	18	33	29	<LOQ	<LOQ	36	<LOQ
P2	<LOQ	140	45	42	70	235	35	41	246	71	<LOQ	<LOQ	97	104	46	328	46	29	37	42	42	21	16	36	

[a] Structurally identified HO-PCBs: **a**, 4-HO-CB 107; **g**, 4′-HO-CB 130; **h**, 3′-HO-CB 138; **i**, 4-HO-CB 146; **j**, 3-HO-CB 153; **k**, 4-HO-CB 162; **p**, 4-HO-CB 187; **s**, 4-HO-CB 193; **t**, 4′-HO-CB 172; **u**, 3′-HO-CB 180. [b] Retention time relative to PCP. [c] The congeners are assigned with letters from **a** to **z** (see also Figure 1) according to their retention time, which is given relative to the retention time of PCP (12.7 min). Detected congeners below LOQ are designated with <LOQ (see Table 3 for LOD and LOQ values). Quantified concentrations are estimated values (see text; given in pg/g ww).

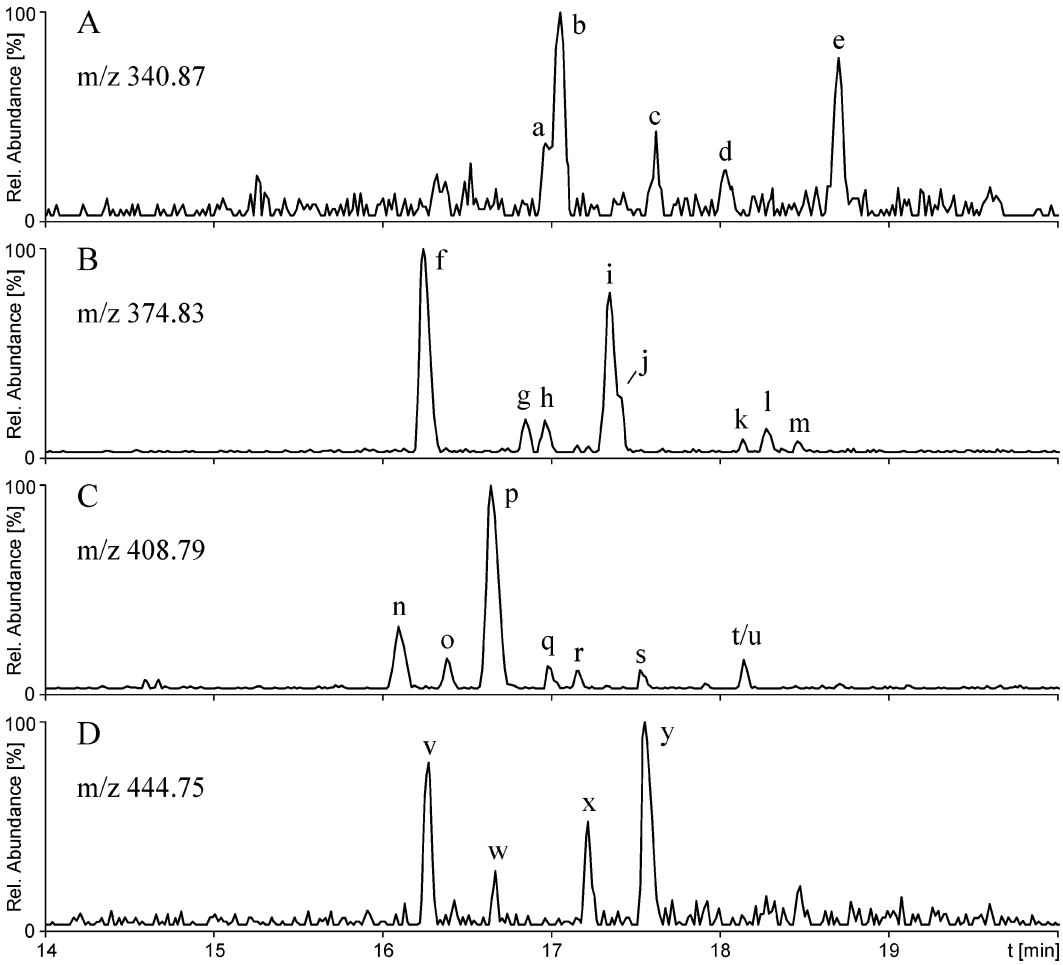

Figure 1. Extracted mass chromatograms for (A) penta-, (B) hexa-, (C) hepta-, and (D) octachloro-HO-PCBs in a peregrine falcon egg sample (P1) analyzed by HPLC/full-scan TOF-MS. Signals assigned with a letter are identified as HO-PCBs. Structurally identified congeners and concentration estimates are given in Table 5.

HPLC/TOF-MS method was determined from the quantification limit (~5 pg injected) to 100 pg injected, corresponding to ~400 pg/g of wet weight (ww) in real samples. In comparison, both GC/MS methods provided linear ranges of at least 4 orders of magnitude for all compounds from the quantification limit upward.

Good recoveries as well as good repeatability were achieved for both validated methods (HPLC/TOF-MS and GC/LRMS), confirming the reliability of the methods including the MCF derivatization prior to GC analysis. Only for TBBPA is there a discrepancy between the recoveries obtained by the two methods. The bulky bromine substituents adjacent to the hydroxyl groups might hamper the derivatization reaction. Another explanation could be the presence of two hydroxyl groups in TBBPA resulting in an incomplete double derivatization. The additional derivatization step in the GC method can also be the reason for the slightly higher relative standard deviations compared to the HPLC method (Table 3). Although the GC/LRMS method is more sensitive than the HPLC/TOF-MS method when employing standard solutions, the opposite was found for quantification of egg sample extracts. This finding can be explained by the high-resolution potential of the TOF-MS efficiently suppressing matrix background in the mass chromatograms. Furthermore, GC/MS in EI mode is highly sensitive for MCF-O-PCBs with a relatively low number of chlorine substituents, whereas the sensitivity of HPLC/TOF-MS toward HO-PCBs increases with the degree of chlorination. The limit of quantification (LOQ = five times blank value) of all methods for PCP in egg samples was found to be ~100 pg/g ww, due to relatively high laboratory blank values (~20 pg/g ww; see also Table 4). The cause of these elevated blank values is unknown so far. GC/HRMS proved to be the only method sensitive enough for analysis of TBBPA in bird eggs.

Recovery rates of the internal standard $^{13}C_{12}$-4-HO-CB 187 in the eight bird of prey egg samples were determined to 58−94 and 64−106% using the HPLC/TOF-MS and GC/HRMS method, respectively. The corresponding recoveries for 4′-HO-CB 26 were 55−72 (HPLC) and 49−95% (GC). $^{13}C_6$-PCP (added to the bird eggs O2, GE2, and SE1) was recovered with 107−117 (HPLC) and 99−111% (GC), and in GC/HRMS analysis its recoveries deviated not more than 5% from the recovery rates of $^{13}C_{12}$-4-HO-CB 187 for individual samples. Therefore, $^{13}C_{12}$-4-HO-CB 187 was used as internal standard for quantification of all compounds of interest (including PCP and TBBPA) in all samples.

HO-PCB congeners with the hydroxy group in ortho position to the interannular phenyl−phenyl bond (2-HO-PCBs) could not be analyzed quantitatively with any of the developed methods. This was due to very low response factors in HPLC/ESI(−)-TOF-MS (at least 100 times lower than for 3- and 4-HO-PCBs) and incomplete derivatization with MCF prior to GC analysis (reaction

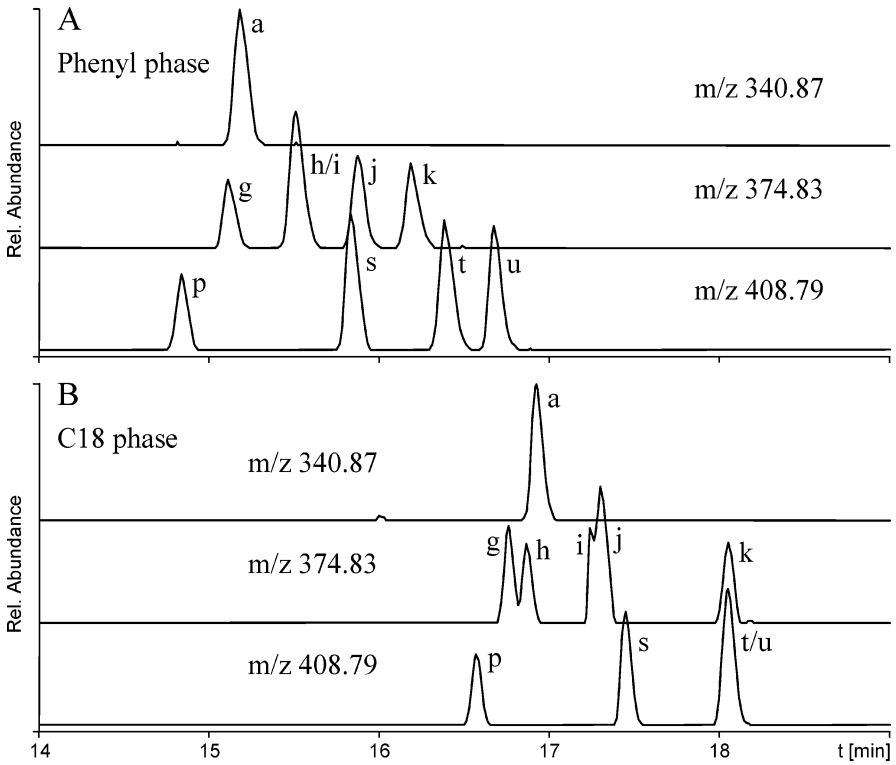

Figure 2. Comparison of the HPLC separation properties for the standard HO-PCBs on the phenyl column (A) and the C_{18} column (B) using the same gradient (see Experimental Section). Extracted mass chromatograms are given for penta- (*m*/*z* 340.87), hexa- (*m*/*z* 374.83), and heptachlorinated (*m*/*z* 408.79) congeners analyzed by HPLC/full-scan TOF-MS. Structure assignment: **a**, 4-HO-CB 107; **g**, 4′-HO-CB 130; **h**, 3′-HO-CB 138; **i**, 4-HO-CB 146; **j**, 3-HO-CB 153; **k**, 4-HO-CB 162; **p**, 4-HO-CB 187; **s**, 4-HO-CB 193; **t**, 4′-HO-CB 172; **u**, 3′-HO-CB 180 (see also Table 5 and Figure 1).

yields estimated to 70−90%). However, there is no 2-HO-PCB congener among 33 structurally identified HO-PCBs in human plasma analyzed after derivatization with diazomethane.[15] The steric shielding of the ortho position in the biphenyl backbone structure impairing ionization by ESI(−) and derivatization with MCF probably also hampers metabolism in this position.

Halogenated Phenolic Compounds in Predatory Bird Eggs. PCP was found in all analyzed bird of prey eggs both by the HPLC/TOF-MS and by the GC/HRMS quantification method (analyzed as MCF-PCP by GC). TBBPA was also found in all samples, but only using the GC method (analyzed as $(MCF)_2$-TBBPA). The HPLC method was not sensitive enough to detect the low levels of TBBPA present in the eggs. The detected PCP and TBBPA levels in the eggs are summarized in Table 4. Due to the relatively low content of extractable organic material in the eggs (1.8−5.5%, determined gravimetrically), all values are given on wet weight bases. As can be seen from Table 4, results for PCP correspond well for the two methods, confirming their robustness. With the exception of one osprey egg sample containing 1.4 ng of PCP/g ww, the levels found both for PCP and for TBBPA are quite low. However, it is noteworthy that TBBPA was found in all samples. Since the predatory birds studied have different feeding habits (Table 1), these findings indicate a ubiquitous distribution of TBBPA in the aquatic and terrestrial environment. Furthermore, it implies that TBBPA can be transferred from the mother organism to the egg during egg formation.

Applying the HPLC/TOF-MS method, 26 penta- to nonachloro-HO-PCB congeners were detected in the bird of prey eggs. They were assigned with letters from **a** to **z** according to their retention times. Their presence and estimated concentrations (see Experimental Section; Recovery Experiments and Quantification) in the different eggs are listed in Table 5. Extracted mass chromatograms for penta- to octachloro-HO-PCBs in a peregrine falcon egg sample (P1) are shown in Figure 1. Ten of the detected congeners could be structurally identified with help from the respective reference standards (see Table 5). Integration was performed using the extracted mass chromatograms recorded after separation on the C_{18} phase HPLC column. Applying this column, the HO-PCBs **a** (4-HO-CB 107) and **b** (identified as the most abundant pentachloro-HO-PCB congener), **i** (4-HO-CB 146), and **j** (3-HO-CB 153) as well as **t** (4′-HO-CB 172) and **u** (3′-HO-CB 180) partly coeluted (see Figure 1). In contrast, the phenyl column completely resolved all of these three couples. The different separation properties of the phenyl and the C_{18} phase columns with respect to the 10 environmentally relevant standard HO-PCBs are shown in Figure 2. From the chromatograms in Figure 2 it can be seen that individual congener quantification was possible for all standard compounds by combining the results obtained from both columns. In the egg extracts, only the signals of 4′-HO-CB 172 and 3′-HO-CB 180 were not abundant enough for reliable individual quantification. However, from the chromatograms of the predatory bird egg extracts recorded by employing the phenyl column, it was obvious that 4′-HO-CB 172 was the dominant of these two congeners. In the mass chromatograms obtained from GC/HRMS analysis, 50 penta- to heptachloro-MCF−O−PCBs were detected, including the 10 structurally identified congeners. They were numbered from **1** to **50** according to their retention times. Their presence and approximate concentrations in the predatory bird

Table 6. Pentachloro to Heptachloro MCF-O-PCBs Detected in the Predatory Bird Eggs by GC/HRMS[c]

	pentachloro MCF-O-PCB																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**[a]	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
rRt[b]	1.55	1.58	1.65	1.70	1.71	1.74	1.76	1.82	1.87	1.97	1.99	2.00	2.03	2.07	2.08	2.10	2.11	2.13	2.15	2.19	2.20
O1			12		15							19						17		<LOQ	
O2					<LOQ							<LOQ			<LOQ			<LOQ			
GE1			13																		
GE2																		<LOQ		<LOQ	
SE1	<LOQ		<LOQ		20		<LOQ	<LOQ			15	<LOQ	<LOQ	12	32	<LOQ	<LOQ	120	13	31	136
SE2	18				36		<LOQ				<LOQ	<LOQ	<LOQ	<LOQ	11	<LOQ	<LOQ	50	<LOQ	20	25
P1				<LOQ	<LOQ	<LOQ		10	16		23	<LOQ	<LOQ	<LOQ		16	10	240		141	32
P2		18			<LOQ	17		<LOQ	21	14	18	12	14	<LOQ		15	<LOQ	243		134	33

	hexachloro MCF-O-PCB															
	22	**23**	**24**	**25**	**26**	**27**	**28**	**29**[a]	**30**[a]	**31**	**32**	**33**[a]	**34**	**35**[a]	**36**[a]	**37**
rRt[b]	1.74	1.76	1.83	1.84	1.84	1.85	1.87	1.89	1.90	1.91	1.95	1.96	1.97	1.98	2.07	2.08
O1	<LOQ	91	16	11	11	<LOQ		<LOQ	16	15	11		<LOQ			<LOQ
O2	<LOQ	<LOQ	<LOQ					<LOQ	<LOQ				<LOQ			
GE1								12	<LOQ	12	<LOQ	<LOQ				<LOQ
GE2			<LOQ					<LOQ	<LOQ		<LOQ					
SE1	10	114	11	<LOQ		<LOQ		<LOQ	26		<LOQ		<LOQ		<LOQ	
SE2	<LOQ	98	13					<LOQ	18	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
P1		13	<LOQ		<LOQ	28	11	36	171		<LOQ	33	115	26	<LOQ	<LOQ
P2		15	<LOQ		<LOQ	37	29	90	322	<LOQ	<LOQ	68	190	52	<LOQ	<LOQ

	heptachloro MCF-O-PCB											
	38	**39**	**40**	**41**	**42**[a]	**43**	**44**	**45**	**46**[a]	**47/48**[a]	**49**	**50**
rRT[b]	1.86	1.98	1.99	1.99	2.00	2.01	2.08	2.10	2.14	2.16	2.23	2.25
O1	15	<LOQ		<LOQ	57							
O2	17				<LOQ							
GE1					22				<LOQ			
GE2					<LOQ	<LOQ			<LOQ	<LOQ		
SE1	15	16		<LOQ	100		<LOQ					
SE2		<LOQ			61		<LOQ					
P1	<LOQ	16	35	50	388	<LOQ	120		<LOQ	45		
P2		<LOQ	50	70	353	<LOQ	99	20	<LOQ	79	64	17

[a] Structurally identified MCF-O-PCBs: **9**, 4-MCF−O−CB 107; **29**, 3-MCF−O−CB 153; **30**, 4-MCF−O−CB 146; **33**, 3′-MCF−O−CB 138; **35**, 4′-MCF−O−CB 130; **36**, 4-MCF−O−CB 162; **42**, 4-MCF−O−CB 187; **46**, 3′-MCF−O−CB 180; **47**, 4′-MCF−O−CB 172; **48**, 4-MCF−O−CB 193. [b] Retention time relative to MCF-PCP. [c] The congeners are assigned with numbers from **1** to **50** (see also Figure 3) according to their retention time, which is given relative to the retention time of MCF-PCP (10.9 min). Detected congeners below LOQ are designated with <LOQ (see Table 3 for LOD and LOQ values). Quantified concentrations are estimated values (see text) for underivatized HO-PCBs present in the eggs (given in pg/g ww).

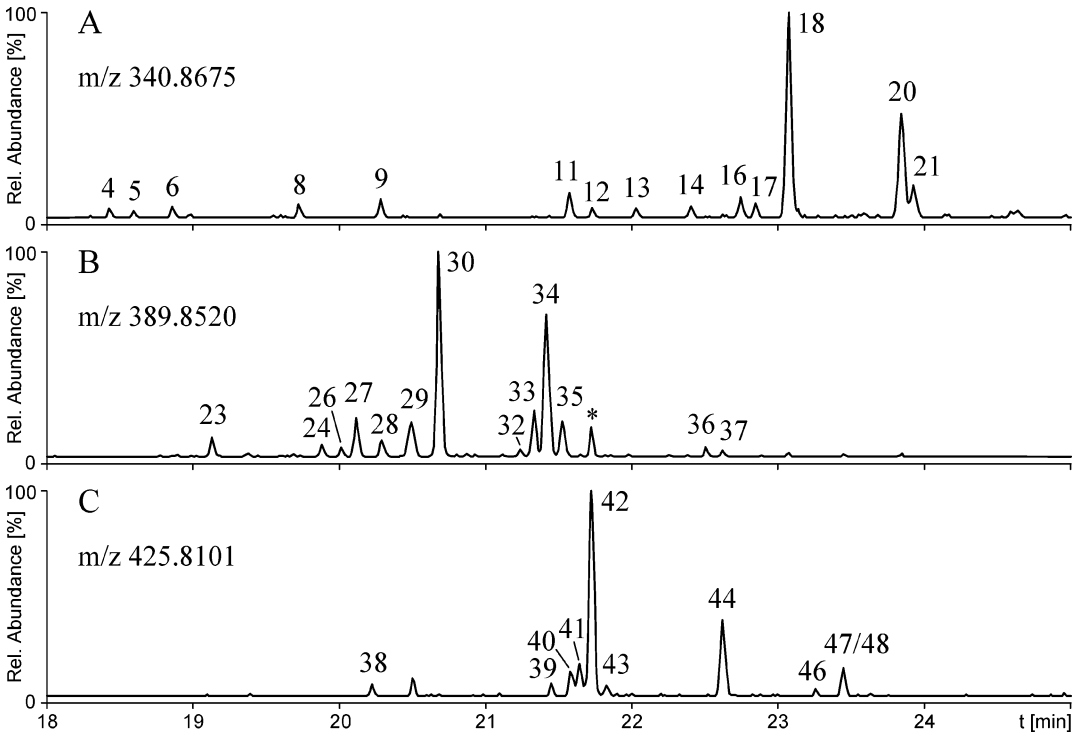

Figure 3. Mass chromatograms for (A) penta-, (B) hexa-, and (C) heptachloro-MCF-O-PCBs in a peregrine falcon egg sample (P1) analyzed by GC/HRMS. Signals assigned with a number are identified as MCF-O-PCBs. The signal in (B) assigned with an asterisk is the fragment $[M - CH_3 - CO_2 - {}^{13}CO]^+$ of the internal standard $^{13}C_{12}$-4-MCF-O-CB 187. Structurally identified congeners and concentration estimates are given in Table 6.

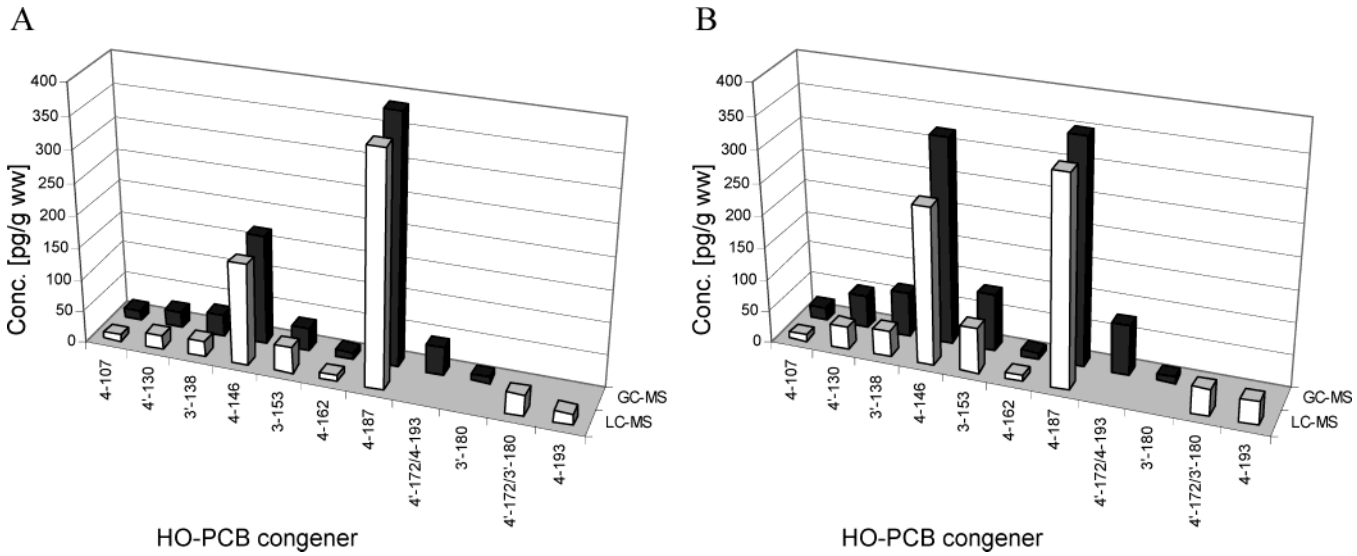

Figure 4. Concentrations of structurally identified HO-PCB congeners in peregrine falcon egg samples P1 (A) and P2 (B). Values are estimated by analysis with HPLC/TOF-MS (white bars) and GC/HRMS (black bars, analyzed as MCF-O-PCBs), respectively. Abbreviations of the congeners are 4−107 for 4-HO-CB 107, etc.

eggs are listed in Table 6. Figure 3 shows the mass chromatograms obtained from an extract of a peregrine falcon egg sample (P1, see Figure 1 for comparison). The heptachloro-MCF-O-PCBs **47** (4′-MCF-O-CB 172) and **48** (4-MCF-O-CB 193) coeluted and could not be quantified separately.

Relating a HO-PCB detected by HPLC/TOF-MS to a MCF-O-PCB signal in the GC/HRMS chromatogram was not feasible for most HO-PCBs, caused by the lack of reference standard of the respective congeners. Therefore, direct comparison of the concentration values obtained from the two methods was only possible for the 10 structurally identified compounds. The levels of these congeners estimated by analysis with the HPLC/TOF-MS and the GC/HRMS method are shown for samples P1 and P2 in Figure 4A and B, respectively. The values obtained by the two methods (white and black bars, respectively) corresponded very well, confirming the reliability of the MCF derivatization and the instrumental methods. In agreement with earlier findings in northern fulmar (*Fulmarus glacialis*) egg samples,[20] 4-HO-CB 187 was the most abundant congener in almost all bird of prey eggs analyzed (maximum concentration found in sample P1; 363 or 388

pg/g ww for HPLC/TOF-MS or GC/HRMS, respectively), followed by 4-HO-CB 146. Compared to blood samples where often 4-HO-CB 107 dominates, the contamination pattern in bird of prey eggs seems to be shifted toward larger (and less water soluble) congeners. It is also noteworthy that in this study four octa- and even one nonachloro-HO-PCB were detected (see Table 5; compounds **v**−**z**). Furthermore, there were two abundant penta-, one hexa-, and one heptachloro-HO-PCB congener present, which could not yet be structurally identified. Due to the relatively high levels of these four unidentified congeners and due to the good correspondence of concentrations found by the two analytical methods, the following relations between unidentified HO-PCBs in HPLC/TOF-MS and MCF-O-PCBs in GC/HRMS chromatograms can be proposed: Congener **b** (HPLC) corresponds to **18** (GC), **e** to **20**, **f** to **34**, and **n** to **44** (see Tables 5 and 6 and Figures 1 and 3). The MCF-O-PCBs **18** and **20** elute very late in the GC chromatogram of pentachloro congeners (Figure 3). However, they were detected with correct exact mass, their abundance ratio of the two analyzed isotope masses corresponded with the theoretical value, and there were also two pentachloro-HO-PCBs (**b** and **e**) detected by HPLC/TOF-MS fulfilling the criteria of accurate mass and correct isotope ratio. Therefore, it can finally be assumed that the signals **18** and **20** represent pentachloro-MCF-O-PCBs.

The HO-PCB concentrations found in the predatory bird eggs are generally quite low. Levels of single congeners did not exceed 400 pg/g ww, and the highest sum HO-PCB concentration was found for sample P2 and estimated to 1.8 or 2.1 ng/g ww by the HPLC/TOF-MS (21 congeners) or GC/HRMS method (27 congeners), respectively. The sum HO-PCBs was in the range of 0.1−1.2‰ of the sum PCB values (18 congeners, data published in detail elsewhere)[37] for the eight investigated samples. These ratios may seem very low. However, it has to be kept in mind that the quantitative results for HO-PCBs given in this study are characterized by a significantly increased uncertainty compared to standard PCB analysis due to the restrictions mentioned in the Experimental Section (see Recovery Experiments and Quantification). In addition, as discussed before (see Results and Discussion, Sample Preparation), it is highly demanding to assess the extraction efficiency for phenolic compounds and it cannot be completely excluded that a significant part of the HO-PCBs remained bound to matrix proteins despite the acidification of the sodium sulfate. Furthermore, large differences in HO-PCB levels between individual eggs were found and a sample set of eight eggs is not sufficiently representative to give an overall picture of the contamination burden of predatory birds and their eggs by HO-PCBs.

Dependency of HO-PCB Congener Pattern on Bird Species. As can be seen from Figure 4, both the concentrations and the contamination patterns of the structurally identified HO-PCBs are similar for the two Peregrine Falcon egg samples. In Figure 5 the relative distribution of the 12 most abundant congeners (five penta-, five hexa-, and two heptachloro-MCF-O-PCBs) from GC/HRMS analysis is shown for all 8 samples. For better visualization, concentration values between LOD and LOQ are used (designated with <LOQ in Table 6). Although only two eggs per species were analyzed, there were comparable congener patterns found for the two samples of the same bird species, whereas distinct differences between species were detected. Of the 12 MCF-O-PCBs shown in Figure 5, the pentachloro congeners (**5**−**21**) make up only about 15−20% in the osprey samples, whereas they constitute 40−50% of the contamination in the white-tailed sea eagle eggs. The hexachloro congener **23** is abundant in osprey and white-tailed sea eagle eggs but neglectable in peregrine falcon samples. Results for golden eagle eggs cannot be interpreted because hardly any HO-PCBs were detectable in these samples. These species-dependent differences might result from different migration and feeding habits of the birds of prey (Table 1) or from different abilities to metabolize PCBs or to transfer PCBs and HO-PCBs from the mother organism to the egg. However, more samples are necessary to confirm or defeat these hypotheses.

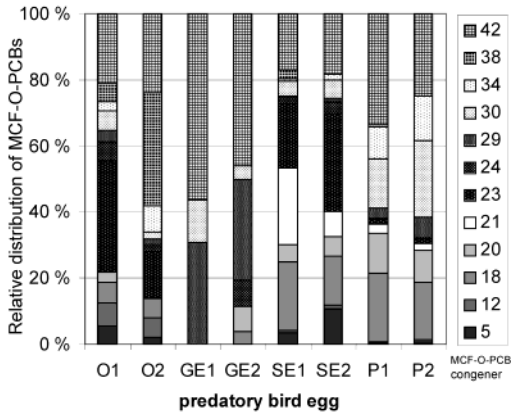

Figure 5. Relative distribution of the 12 most abundant MCF-O-PCB congeners (five pentachloro (**5**−**21**, full gray scale), five hexachloro (**23**−**34**, dotted), and two heptachloro (**38** and **42**, checkered)) from GC/HRMS analysis in all bird of prey egg samples (see Table 6 and Figure 3 for congener identification). For better visualization also concentration values between LOD and LOQ are used (designated with <LOQ in Table 6).

CONCLUSIONS

Two new trace analytical methods for identification and quantification of phenolic compounds in complex biological matrixes such as bird eggs were developed. To our best knowledge, these are the first HPLC/MS method dealing with environmental analysis of hydroxylated PCBs and the first method employing methyl chloroformate as derivatization reagent for HO-PCBs prior to GC separation. Both methods are well suited for analysis of pentachlorophenol, tetrabromobisphenol A, and hydroxylated PCBs at levels down to few picograms per gram of wet weight in abandoned predatory bird eggs. The advantages of the HPLC/TOF-MS method are that no derivatization of phenolic compounds is necessary prior to separation and the simultaneous detection of all analytes without sensitivity loss by full-scan TOF-MS. The GC/HRMS method showed better separation properties and was slightly more sensitive than the HPLC method, especially for TBBPA. Furthermore, applying two complementary methods is recommended as a quality assurance measure for analysis of HO-PCBs due to the high number of hitherto unidentified congeners, possible coelution problems, and lack of authentic reference standards leading to uncertainty in quantification. Extraction and derivatization proved to be the crucial steps in

(37) Herzke, D.; Berger, U.; Nygård, T.; Vetter, W. *Organohalogen Compd.* **2003**, *61*, 466−469.

sample preparation, and further optimization of these processes must be performed. High-resolution mass spectrometry is advantageous and can even be mandatory for analysis of trace amounts of phenolic compounds in complex environmental matrixes.

The predatory bird eggs analyzed in this study showed varying levels of PCP and low concentrations of TBBPA and HO-PCBs. PCP and TBBPA were found in all investigated samples, confirming the ubiquitous presence of these contaminants in the aquatic and terrestrial environment. Furthermore, a total number of 55 penta- to nonachloro-HO-PCBs were detected by the two methods, causing concern and showing an urgent need for further research work in this field. Peregrine falcon eggs (known to contain high concentrations of PCBs and organochlorine pesticides)[27,37] were the highest contaminated with individual HO-PCB congeners as well as sum HO-PCBs. Indications were found that the HO-PCB congener pattern could be species specific for predatory birds. However, this hypothesis can only be verified by analysis of a representative number of additional bird of prey eggs.

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NOTE ADDED AFTER ASAP

The article was posted on the Web on 12/9/03. Subsequently, two typographical errors in concentrations were corrected, and the article was reposted on 12/18/03.

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