Screening chlorinated paraffins in Swedish terrestrial birds and mammals (2012-2017)

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Aim: This is a screening study commissioned by the Swedish EPA within the national environmental monitoring program. The aim is to study occurrence of a group of emerging contaminants, i.e., short-chain, medium-chain, and long-chain chlorinated paraffins, in Swedish terrestrial mammals and birds collected within the past five years. The species include both predators and prey species, in order to test whether these chemicals possess bioaccumulation potential through the terrestrial food chain.

Introduction. Chlorinated paraffins (CPs) are widely-used lubricants, plasticizers, flame retardants, and metal cutting fluids.[1] CPs refer to complex mixtures of polychlorinated n-alkane chains, from n-hexane to n-octatriacontane (i.e., C_6−C_{38} CPs).[2] The commercial products are categorized into short-chain (≤ C_{13}, SCCPs), medium-chain (C_{14} – C_{17}, MCCPs) and long-chain (≥C_{18}, LCCPs) products according to the carbon-chain-length distribution. These products are further subcategorized according to their chlorine contents (30 – 70 %Cl by weight).[3, 4] CPs with identical numbers of carbon and chlorine atoms are defined as one congener group, denoted as C_nCl_m.[5] Today, the annual global production exceeds 1 million tons, most of which (ca. 90 %) is in China[1]. In Sweden, CPs are only imported. About 4 800 tons of CPs were imported in the year 1991. Since then, the use of CPs in Sweden has decreased by 80%.[6] However, the amount of CP importation from many products containing CPs has not been taken into account, such as CPs in baking ovens[7] and hand blenders.[8] In 1993, Jansson et al.[9] reported total CPs in pooled rabbit, moose, reindeer, and osprey samples in Sweden, but compositions of SCCPs/MCCPs/LCCPs or C_nCl_m patterns in these were unknown. Our recent study showed that MCCPs and LCCPs are the predominant CPs in use in recent years, and C_8−C_{36} CPs were present in Swedish sediments since the 1930s.[10] To better understand CP contamination in Swedish terrestrial wildlife, we studied the concentrations and C_nCl_m patterns of CPs in four terrestrial mammals and five birds, collected from south-western Sweden in the past five years (between 2012 and 2017).

Methods. The muscle tissues of ten individuals of each species were pooled and analyzed. CP analysis was performed using atmospheric-pressure chemical ionization quadrupole time-of-flight mass spectrometry (APCI-QTOF-MS). All concentrations were above the method detection limits (MDLs). Mean and standard deviation of recoveries of the isotopically-labelled internal standards were 80% ± 11%. Detailed sample and analysis information are given in *Appendix A* and *B*, respectively.

Terrestrial Mammals. Concentrations and chlorine contents of SCCPs, MCCPs, and LCCPs are shown in *Table 1* and *Figure 1*. The C_nCl_m patterns of CPs are shown in *Figures 2* and *3*. The longest CPs observed in terrestrial mammals were C_{28} (*Figure 2*). SCCP, MCCP, and LCCP concentrations in terrestrial mammals (moose, lynx, wolf, and bank vole) in this report ranged from 420-1500, 370-1600, and 36-180 ng/g lipid, respectively (*Table 1*). SCCP, MCCP, and LCCP concentrations were similar in lynx and wolf, but lower in bank voles, indicating possible bioaccumulation of these CPs in the food chain. The concentrations in Swedish terrestrial mammals were significantly lower than those in yellow weasel[11] from the Yangtze River Delta, China (the only mammal reported in that study; median 8900, 12000, and 740 ng/g lipid, respectively). The highest concentrations of SCCPs, MCCPs, and LCCPs were all observed in moose. This is similar to the results of the screening study in 1993, in which moose showed the highest total CP concentrations (4400 ng/g lipid). The total CP concentration in moose muscle from animals collected 2012-2017 reported here were lower (3400 ng/g lipid), indicating possible temporal declines in the environment. A similar decline in the total concentration of CPs was also seen in a coastal sediment core at Himmerfjärden.[10]. The reason for higher concentrations of SCCPs, MCCPs, and LCCPs in moose is unclear. Given that they are the only large herbivores in this report, one possible reason may be their dietary exposure to vegetation such as small deciduous tree branches and leaves, coniferous tree branches, pine bark and shoots, and so on. Accumulation of SCCPs has been reported in

pine needles and bark.[12, 13] CPs might have been accumulated in and/or on vegetation as well, given their similar physical and chemical property to OCPs and PCBs. However, data for CP concentrations in outdoor air or atmospheric deposition, soil, and vegetation in Sweden are not available at present, which requires further study.

Terrestrial Birds. SCCPs, MCCPs, and LCCPs in birds (eagle owl, marsh harrier, golden eagle, peregrine falcon, and starling) in this report ranged from 230-750, 180-720, and 25-1200 ng/g lipid, respectively (*Table 1*). Compared with the terrestrial mammals, bird species accumulated higher concentrations of LCCPs (*Figure 1*). The longest CPs observed in birds were C_{30} (*Figure 3*).The birds of prey (eagle owl, golden eagle, and peregrine falcon) showed higher concentrations of SCCPs, MCCPs, and LCCPs than found in starling and bank vole, indicating CP bioaccumulation in the food chain. The only exception is marsh harrier, which feeds on small mammals including bank voles but showed lower concentrations of SCCPs and MCCPs than those in bank voles. One possible explanation is that CP bioaccumulation is age-related and most marsh harrier samples in this report were juvenile individuals due to lack of adult samples.

High Levels of LCCPs in Birds. The birds of prey (all the bird species except starling) in this report generally accumulated higher concentrations of LCCPs than terrestrial mammals, in particular the peregrine falcon. SCCPs, MCCPs, and LCCPs in peregrine falcon were reported in the Yangtze River Delta, China, in the ranges of 840-14000, 1300-29000, and 530-10000 ng/g lipid, respectively.[11] SCCPs and MCCPs in peregrine falcon in this report were both lower than the lowest values in China; however, LCCPs in peregrine falcon in this report (1200 ng/g lipid) were higher than the median value (690 ng/g lipid) of those in China. LCCPs were also found to predominate in office dust samples from Stockholm (mean 87% of total CPs), which were higher than in office dust from Beijing, China (mean 39% of total

CPs)[14] and this may indicate higher use of LCCPs in Sweden than China. As for the higher concentrations of LCCPs found in peregrine falcon compared to the other predatory birds, this may be due to differences in their diets, in their metabolism, or the fact that peregrine falcons migrate to southern parts of Europe in the winter and they and/or their prey may be more exposed there. Peregrine falcons feed exclusively on birds whereas the other predatory birds feed more on small mammals for example. To determine why birds of prey in Sweden accumulate high levels of LCCPs, studies of CP concentrations in their food chains are needed.

Comparison to Other POPs in Peregrine Falcon. Most populations of peregrine falcon were previously endangered in the northern hemisphere because of the bioaccumulation of high concentrations of several organochlorine pesticides and mercury, which affected both reproduction and survival.[15] In this report the highest concentrations of LCCPs were identified in this species, which might be a new possible threat to them. Several studies reported high concentrations of higher brominated BDEs including BDE-209 in Swedish peregrine falcon eggs.[15-17] Here we compared CP concentrations in Swedish peregrine falcon muscle (2012-2016) with some other POPs or POP-like contaminants in their eggs collected in the same decade (2007). Although few data are available for muscle samples, contaminant concentrations in eggs can indicate the levels in female individuals. As shown in Table 2, LCCP concentrations in peregrine falcon muscle from 2012-2016 are comparable to ∑PBDEs in eggs from 2007.[16] Total CP concentrations in the muscle samples are lower than ∑PCBs and p,p'-DDE (unpublished data) in the eggs by a factor of 10-20, but higher than HBCD in eggs[16] by a factor of 13.

Conclusion. There are at least three notable indications from these results: 1) CPs up to C_{30} are widely present in the Swedish terrestrial environment; 2) CPs, especially MCCPs and

LCCPs, have bioaccumulation potential through the terrestrial food chain; 3) Swedish birds, especially peregrine falcons, are more significantly exposed to LCCPs than terrestrial mammals.

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Table 1. Concentrations and chlorine contents of chlorinated paraffins in Swedish terrestrial mammals and birds (2012-2017).

group	***species***	***Concentration (ng/g lipid weight)***				***Chlorine content (weight/weight)***			
		Total	***SCCPs***	***MCCPs***	***LCCPs***	***Total***	***SCCPs***	***MCCPs***	***LCCPs***
mammals	moose / älg	3400	1500	1600	180	51%Cl	57%Cl	50%Cl	43%Cl
	lynx / lodjur	1700	820	750	92	52%Cl	58%Cl	50%Cl	40%Cl
	wolf / varg	2000	1100	830	100	52%Cl	57%Cl	51%Cl	42%Cl
	bank vole / skogssork	820	420	370	36	52%Cl	56%Cl	50%Cl	44%Cl
birds	eagle owl / berguv	1800	750	720	380	54%Cl	61%Cl	53%Cl	47%Cl
	marsh harrier / brun kärrhök	520	230	180	100	52%Cl	58%Cl	53%Cl	46%Cl
	golden eagle / kungsörn	1100	570	360	210	54%Cl	61%Cl	53%Cl	46%Cl
	peregrine falcon / pilgrimsfalk	2100	580	410	1200	53%Cl	63%Cl	57%Cl	48%Cl
	starling / stare	690	360	310	25	51%Cl	56%Cl	50%Cl	42%Cl

Table 2. Comparison of different POPs/POP-like contaminants accumulated in peregrine falcons and their eggs in Sweden in the past decade.

Matrix	Year	Location	Contaminants	Concentration (ng/g lipid)	Reference
muscle	2012-2016	Southern-middle Sweden	∑CPs	2100	This study
			SCCPs	580	
			MCCPs	410	
			LCCPs	1200	
eggs[a]	2007	Southern Sweden	∑PCBs[b]	35000	(unpublished data)
			p,p'-DDE	27000	(unpublished data)
			∑PBDEs[c]	1300	Johansson et al. (2011)[16]
			HBCD	160	Johansson et al. (2011)[16]
			∑PFOS	47[d]	Holmström et al. (2010)[18]
			∑PFAS	62[d]	Holmström et al. (2010)[18]

a. Pool of five individuals.
b. Sum of seven congeners.
c. Sum of BDE47, 99, 100, 153, 183, and 209.
d. Unit: ng/g wet weight.

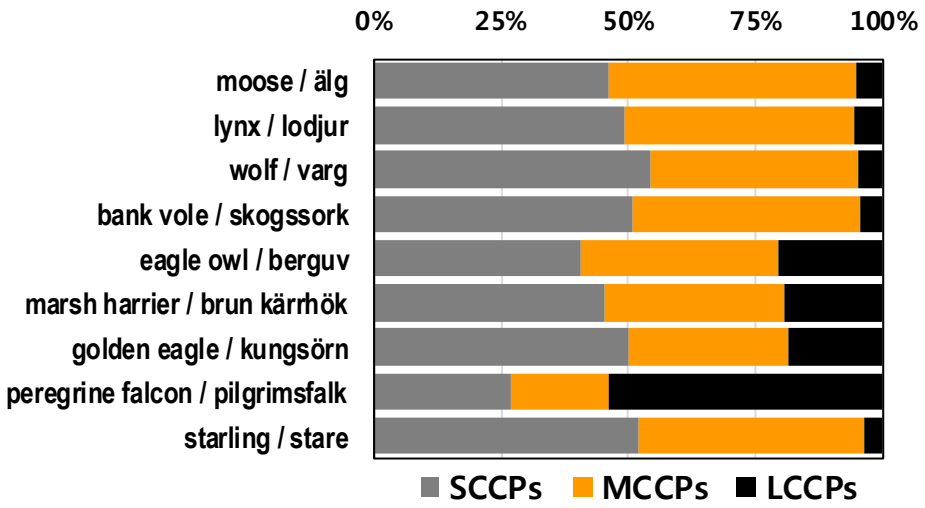

Figure 1. Percentage compositions of SCCPs, MCCPs, and LCCPs in terrestrial mammals and birds.

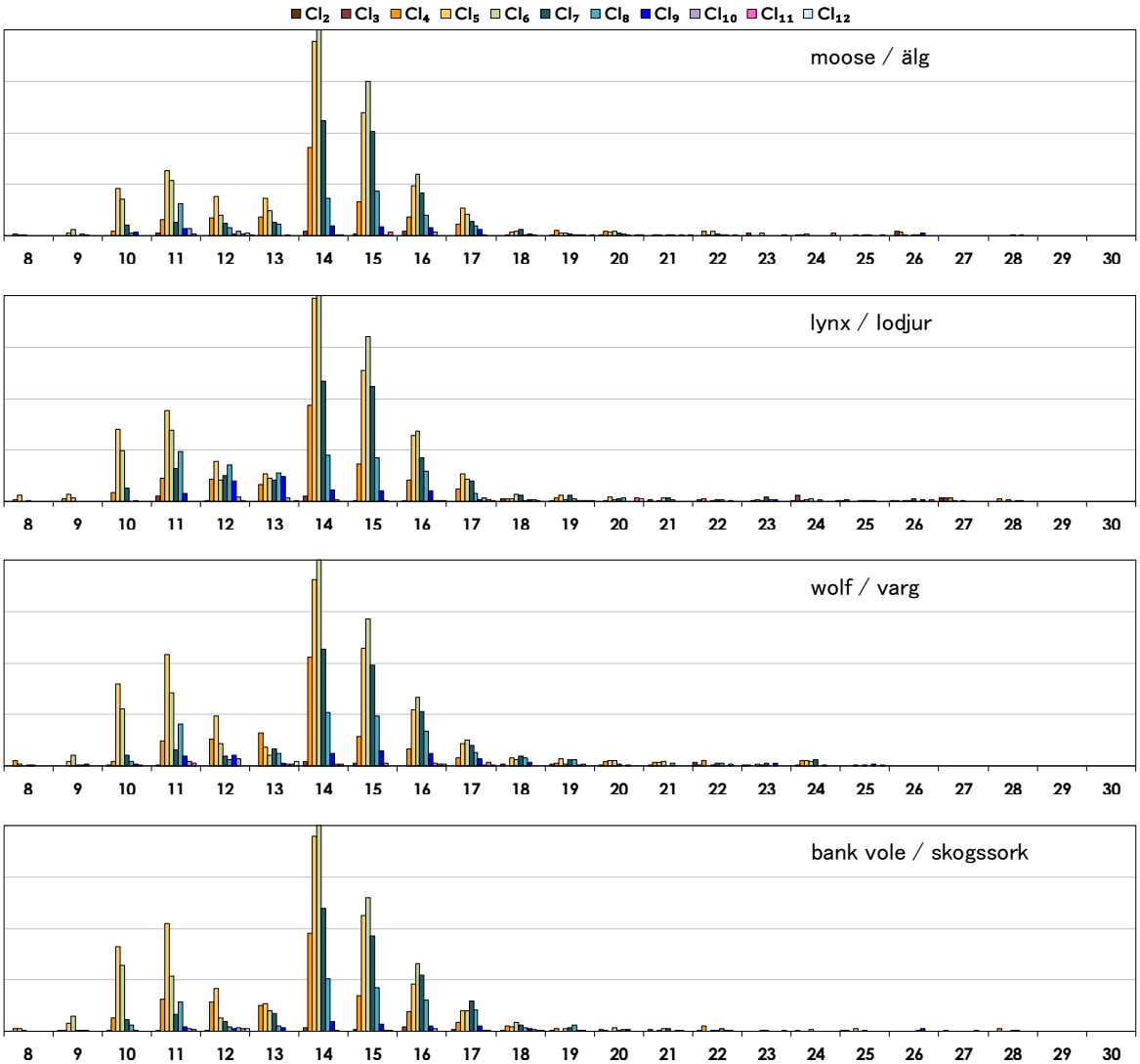

Figure 2. C_nCl_m patterns in the terrestrial mammal samples. X-axis represents carbon chain lengths. Y-axis represents relative abundances of individual congener groups.

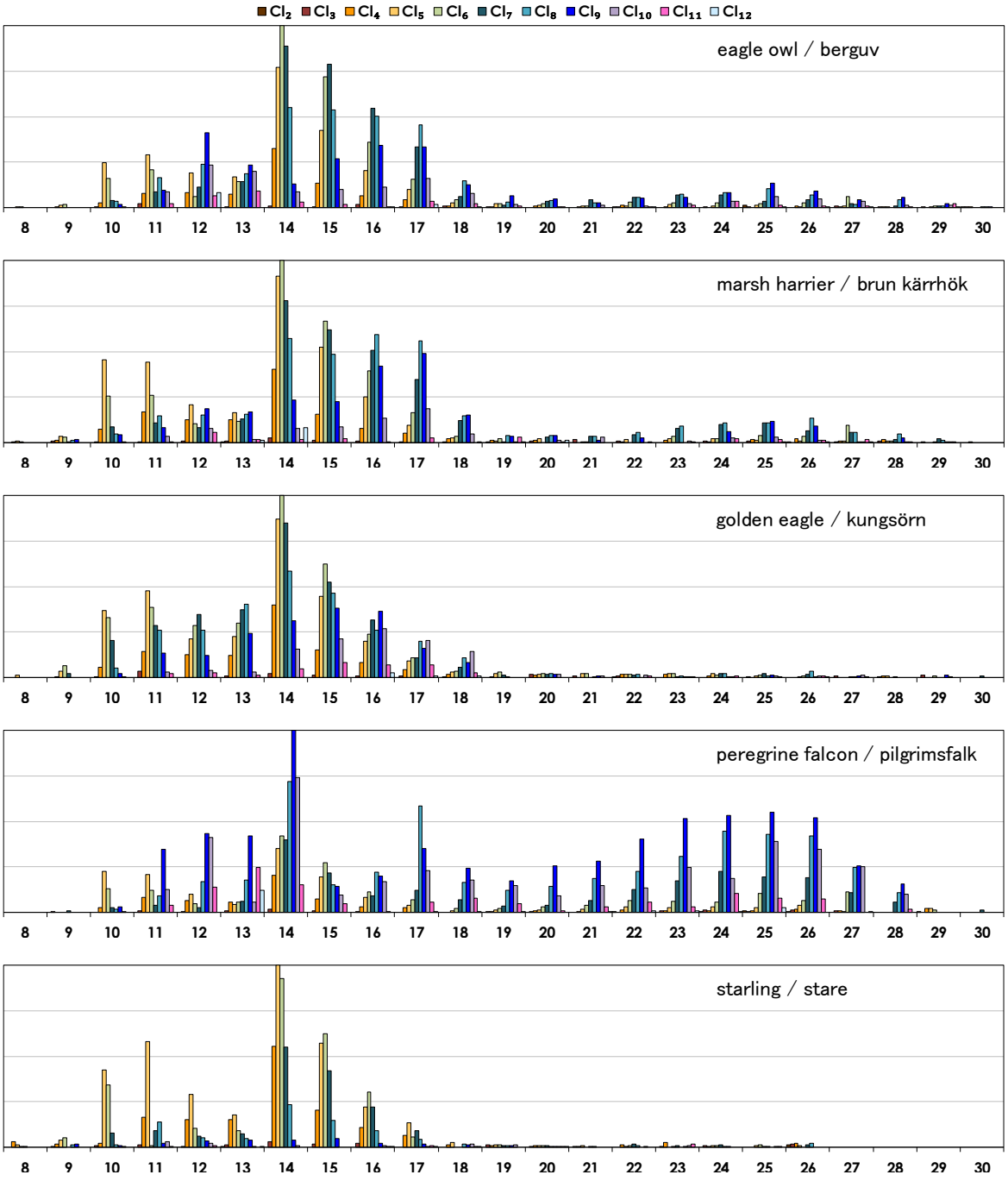

Figure 3. C_nCl_m patterns in the bird samples. X-axis represents carbon chain lengths. Y-axis represents relative abundances of individual congener groups.

Appendix A: Sample Information

The samples in this report were collected and pooled by Naturhistoriska Riksmuseet. The muscle tissue of ten individuals of one species were pooled (*Table A*), which was approximately 2-gram fresh muscle from each individual. The individuals were pooled according to gender and sampling year. The pooled individuals were generally adults of each species, except for marsh harrier (brun kärrhök), which were mainly juvenile individuals due to lack of adult samples. The pooled samples were stored in glass containers in a -20 ℃ freezer until analysis.

Table A. Sample information.

Type	*Species*	*Region*	*Sample size*	*water content%*	*lipid content%*	*Years*	*Feeding habits*	*Recovery of internal standard*
Mammals	moose / älg	Västmanland	10	77%	0.6%	2012-2015		82%
	lynx / lodjur	Västmanland	10	75%	1.4%	2012-2016	feed on vole, fox, and etc.	95%
	wolf / varg	Västmanland	10	75%	1.4%	2012-2016	feed on moose and etc.	82%
	bank vole / skogssork	Värmland	10	73%	3.8%	2014		85%
Birds	eagle owl / berguv	south-middle	10	74%	1.5%	2013-2017	feed on small mammals including vole	80%
	marsh harrier / brun kärrhök	south-middle	10	72%	4.6%	2012-2015	feed on small mammals including vole and frog	73%
	golden eagle / kungsörn	south-middle	10	71%	4.8%	2012-2016	feed on medium mammals including rabbits and birds	78%
	peregrine falcon / pilgrimsfalk	south-middle	10	74%	2.7%	2012-2016	feed on medium-sized birds	57%
	starling / stare	south-middle	10	77%	3.0%	2012-2015	feed on invertebrates including insects and worms	90%

Appendix B: Analytical Method

B.1. Extraction and clean-up

The extraction and cleanup process was adopted from previous studies.[19-21] Prior to extraction, the freeze-dried samples were spiked with 10 ng of $^{13}C_{10}$-1,5,5,6,6,10-hexachlorodecane as the internal standard. The extracts were condensed and then cleaned-up on a multilayer SPE column packed with 2 g silica (deactivated with 2.5% H_2O), 8 g 44% sulfuric acid silica and 4 g of anhydrous sodium sulfate from bottom to top. The concentrated extract was loaded and eluted using 30 mL of hexane and 15 mL of hexane/dichloromethane (1:1, v/v). The second eluent was concentrated and solvent-exchanged to isooctane.

B.2. Reference standards

A set of 47 commercial CP products and reference standards were analyzed initially, of which 16 (5 SCCPs, 6 MCCPs and 5 LCCPs) were selected for quantifying samples in this report. The selected standards are shown in *Table B.*

B.3. Instrumental analysis

CPs were measured using APCI-QTOF-MS. CP congener groups from C_8Cl_3 to $C_{30}Cl_{12}$ were considered to form a congener group pattern. The contribution of each CP congener group was the sum of two instrument responses corresponding to the two *m/z* ratios of the congener group. Total response of CPs was the sum of the contributions from the individual congener groups. Congener group patterns as well as total response factors of the selected products were measured for quantification. The recovery of ^{13}C-labelled CP congener standard was measured using a GC-MS.

B.4. Quantification procedure/Pattern deconvolution

CP response factor of each sample was calculated by a deconvolution algorithm which has been given in Bogdal et al.[22] The CP congener group pattern of each sample was reconstructed from CP patterns of the selected standards. The reconstructed pattern was compared to the initial pattern of the analyzed sample to determine the goodness of fit (R^2). CP patterns in all samples were satisfactorily deconvoluted (i.e. $R^2 \geq 0.50$). Pattern reconstruction of CPs in the golden eagle sample is shown in *Figure B* as an example. Relative contributions of the standards (*Table B*) were then used to calculate instrument response factors of SCCPs, MCCPs and LCCPs in the sample.

B.5. QA/QC

Sample preparation was performed in a vertical laminar flow hood. Mean and standard deviation of recoveries of the ^{13}C-labelled CP congener standard were 80% ± 11% (*Table A*). SCCPs and MCCPs in the procedural blank were 4.8 ng and 1.1 ng, respectively. The method detection limits (MDLs) of SCCPs and MCCPs were defined as three times the procedural blank. No LCCPs were observed in the procedural blank. Therefore the instrumental LOQ of LCCPs (0.9 ng when the signal-to-noise ratio was 10:1) was used to calculate the method detection limits (MDLs) of LCCPs. These quantities were converted to concentrations by dividing by 0.3 g lipid, which was the average lipid weight of individual extracts in this report. The MDLs of SCCPs, MCCPs, and LCCPs in this report were 48, 11, and 3 ng/g lipid, respectively.

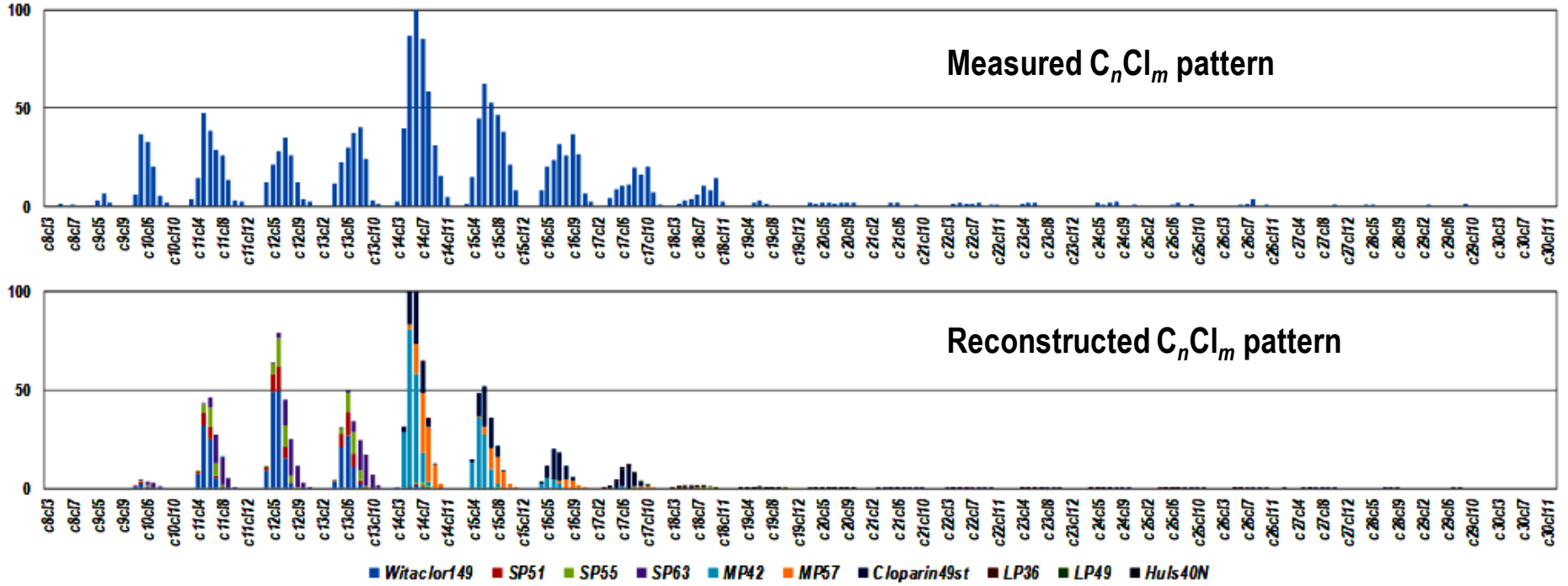

Figure B. C_nCl_m pattern reconstruction in the golden eagle sample (R^2 = 0.86). Relative contributions of technical CP products are given in *Table B.*

Table B. Relative contributions of chlorinated paraffin standards in pattern reconstruction of individual pooled samples.

species	R^2	*Relative contributions from SCCP products*					*Relative contributions from MCCP products*						*Relative contributions from LCCP products*				
		Witaclor 149	*SCCP 51.5% Cl*	*SCCP 55.5% Cl*	*SCCP 63% Cl*	*Hüls 70C*	*MCCP 42% Cl*	*MCCP 52% Cl*	*MCCP 57% Cl*	*Cloparin 49st*	*Cloparin 50*	*Cereclor S52*	*LCCP 36% Cl*	*LCCP 49% Cl*	*Uniclor 40*	*Hüls 40N*	*Witaclor VP549*
moose / älg	0.87	32%		0%	1%	2%	43%		0%	13%	0%	8%	0%	0%		0%	1%
lynx / lodjur	0.85	31%			5%		44%		3%	15%		0%	1%				0%
wolf / varg	0.82	36%			2%	1%	42%		4%	15%		0%	1%			0%	0%
bank vole / skogssork	0.81	35%			1%	0%	44%		4%	15%		0%	1%				0%
eagle owl / berguv	0.82	3%	9%	2%	4%	15%	8%		9%	30%	0%	1%		1%	7%		10%
marsh harrier / brun kärrhök	0.78	3%	16%		6%	3%	36%	0%	9%		27%		0%	0%	0%	0%	0%
golden eagle / kungsörn	0.86	21%	5%	6%	8%		34%		13%	11%			0%	0%		0%	
peregrine falcon / pilgrimsfalk	0.50	5%			7%	26%	14%		15%		0%			3%	12%		18%
starling / stare	0.79	35%			1%	0%	49%		3%	10%		0%	1%				

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