Distribution and Elimination of Polychlorinated Dibenzo-*p*-dioxins, Dibenzofurans, Biphenyls, and *p,p′*-DDE in Tissues of Bald Eagles from the Upper Peninsula of Michigan

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Liver, muscle, fat, kidney, and gall bladder of eight bald eagles (*Haliaeetus leucocephalus*) found dead in the Upper Peninsula of Michigan during 2000 were analyzed for the presence of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (including coplanar PCBs), *p,p′*-DDE, and hexachlorobenzene (HCB). Necropsy results showed that the birds suffered from peritonitis, bacterial infection, or trauma. Concentrations of PCDD/DFs in livers ranged from 23 to 4500 pg/g on a wet weight basis (wet wt), whereas the least concentrations were found in blood plasma of bald eagle nestlings (2.3−49 pg/g, wet wt). A maximum total PCB concentration of 280000 ng/g, wet wt, was found in the liver of a dead bald eagle affected by peritonitis. The greatest concentrations of *p,p′*-DDE and HCB in eagle livers were 17000 and 120 ng/g, wet wt, respectively. Eagles with elevated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or total PCB concentrations tended to have great TCDD/TCDF or PCB126/PCB77 ratios, hypothesized to be due to induction of cytochrome P450 enzymes and subsequent metabolism of TCDF and PCB77. Concentrations of TCDD toxic equivalents (TEQs) in the tissues of bald eagles exceeded the thresholds for toxicity in a few avian species. Non-*ortho* coplanar PCBs accounted for 68−88% of the total TEQs in bald eagle tissues. PCDDs and PCDFs collectively accounted for, on average, 17% of the total TEQs. On the basis of the analysis of a single gall bladder with bile, biliary excretion rates of PCDDs, PCDFs, and PCBs were estimated as 0.015−0.02% per day.

Introduction

Bald eagles are predatory birds, which feed at the top of the aquatic food web. Because bald eagles are relatively long-lived birds (up to 30 years in the wild), they remain sensitive to environmental toxicants that bioaccumulate and affect critical biological processes such as reproduction. Several studies have examined concentrations of persistent chlorinated hydrocarbon contaminants such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) in tissues of bald eagles (*1*−*10*). Some of these studies have suggested a causal relationship between reduced reproductive success of bald eagles and exposures to DDT and its metabolite, DDE, and PCBs. Regulation of the use of DDT in the early 1970s has resulted in the recovery of bald eagle populations in the United States, although, in some locations, PCBs and other chlorinated hydrocarbons continue to affect bald eagle production (*9*). This remains a cause for concern in certain areas, notably in the Great Lakes region. The bald eagle population in Michigan has increased in the past several years, although at a slower rate than those in other regions (*2*, *11*−*13*). Only a few studies have reported concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in bald eagles from Michigan (*3*, *11*, *12*). Previous studies have reported concentrations of PCBs and DDE in the plasma of eaglets or addled eggs (*3*, *11*, *12*). Reports of PCB or DDT concentrations in bald eagle tissues such as liver, muscle, fat, kidney, or gall bladder are sparse due to the difficulties in obtaining such tissues. Because the bald eagle was listed as an endangered/threatened species in the United States until 1999, invasive sampling of tissues from live birds has been limited, prohibited, or controlled. Therefore, only dead or accidentally killed individuals were used for monitoring contaminant concentrations in internal organs such as liver or kidney. In this study, tissues of freshly dead bald eagles were collected from the Upper Peninsula of Michigan. This provided an opportunity to analyze chlorinated hydrocarbons in several body tissues. Analyses of PCDDs and PCDFs in tissues would provide information on the magnitude of toxicity, estimated as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalents (TEQs), which can be compared with those contributed by PCBs. Considering the lack of information on tissue-specific accumulation of PCDDs, PCDFs, dioxin-like and total PCBs, *p,p′*-DDE, and HCB, concentrations of these compounds were measured in liver, kidney, muscle, fat, blood plasma, and gall bladder of bald eagles collected from the Upper Peninsula of Michigan. Analysis of several tissues would provide information on the partitioning of organochlorines among body tissues including physiologically important organs such as the liver and kidneys. This information can be used to calculate concentration quotients, which are useful in risk assessments. TEQs were calculated using the World Health Organization (WHO) toxic equivalency factors (TEFs) for birds (*14*) and compared with thresholds for toxicity reported in the literature.

Materials and Methods

Sample Collection. Carcasses of bald eagles that were found dead in the Upper Peninsula of Michigan in 2000 were collected by or submitted to the Rose Lake Wildlife Research Center, Lansing, MI. The carcasses were transported to the wildlife research center and, upon receipt, they were necropsied and cause of death was determined. Samples of liver, muscle, fat, kidney, and gall bladder (those that were in good condition) were wrapped in solvent-clean aluminum foil and stored at −20 °C until analysis. The gall bladder filled with bile was also analyzed. In addition, blood plasma of nestling bald eagles (approximately <60 days old), collected

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TABLE 1. Details of Bald Eagle Samples Employed in This Study

sample code	date of collection	tissue[a]	age	sex	location	wt (kg)	diagnosis
WDL 373	May 25, 2000	GB, M, L	adult	male	Cecil Bay	3.3	peritonitis, bacterial infection
WDL 374	April 26, 2000	L	adult	male	Ironwood	4.0	trauma (car kill?)
WDL 377	April 27, 2000	K, M	juvenile	female	Mackinac	3.3	peritonitis; airsacculitis
WDL 378	February 4, 2000	L, F, M	juvenile	female	Menominee County	4.2	trauma (gunshot?)
WDL 379	May 31, 2000	M, L	adult	female	Paint River	4.9	trauma
WDL 380	March 29, 2000	F	juvenile	female	Baraga	4.5	trauma (car kill?)
WDL 381	March 30, 2000	K,L,M	adult	male	Fence River	2.3	starvation, trauma (old)
WDL 382	May 4, 2000	L, M	juvenile	female	Kallio	3.5	poisoning (lead?)
P-113	June 27, 1993	plasma	chicks; <60 days	NA[b]	Iron Lake	NM[c]	
P-094	June 20, 1993	plasma	chicks; <60 days	NA	King Lake	NM	
P-084	June 18, 1993	plasma	chicks; <60 days	NA	Seney	NM	
P-096	June 20, 1993	plasma	chicks; <60 days	NA	Slate River	NM	

[a] GB, L, K, M, and F represent gall bladder, liver, kidney, muscle, and fat, respectively. Gall bladder was filled with bile. [b] NA, not analyzed. [c] NM, not measured.

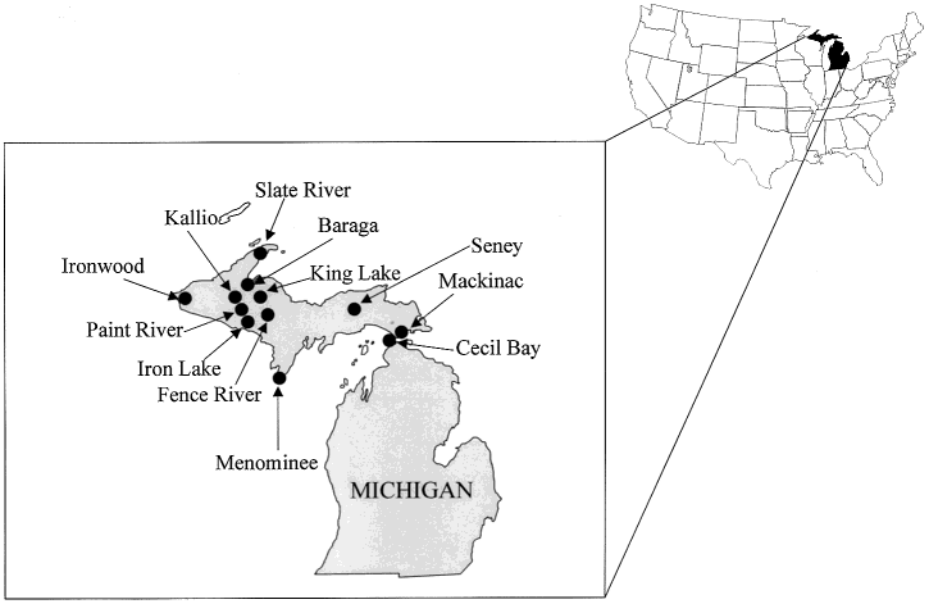

FIGURE 1. Map of Michigan showing sampling locations of bald eagles.

during 1993 were also analyzed. To separate blood plasma, ~10 mL of whole blood was centrifuged and stored at −20 °C until analyzed. Details of the samples analyzed in this study are shown in Table 1, and sampling locations are shown in Figure 1.

Chemical Analysis. Prior to analysis, all of the tissues and plasma were freeze-dried, and the moisture content was determined. Liver, muscle, and fat were extracted in dichloromethane (DCM) using a Soxhlet apparatus for 10−15 h, whereas plasma, gall bladder, and kidney were extracted in DCM using an accelerated solvent extractor. Details of the analytical procedure have been reported previously (*15*−*17*). Briefly, after extraction, samples were concentrated to 10 mL using a Kuderna-Danish (K-D) concentrator, and the solvent was transferred to *n*-hexane. Lipid content was determined gravimetrically from an aliquot of the extract. Seventeen ^{13}C-labeled 2,3,7,8-substituted tetra-, penta-, hexa-, hepta-, and octa-CDD and CDF congeners and 14 dioxin-like PCBs (IUPAC No. 81, 77, 126, 169, 105, 114, 118, 123, 156, 157, 167, 170, 180, and 189) were spiked into hexane extracts prior to sulfuric acid treatment. The hexane layer was rinsed twice with hexane-washed water and dried by passage through anhydrous sodium sulfate in a glass funnel. The solution was concentrated to 2 mL and sequentially subjected to silica gel, alumina, and silica gel impregnated activated carbon column chromatography. Extracts were passed through activated silica gel (activated at 130 °C for 3.5 h) packed in a glass column (Wakogel, silica gel 60; 2 g) and eluted with 210 mL of hexane. This eluant contained PCDD/DFs, PCBs, *p*,*p*′-DDE, and HCB. This was K-D-concentrated and passed through an activated alumina (190 °C for 3 h) column (Merck-Alumina oxide, activity grade 1; 5 g) and eluted with 30 mL of 2% DCM in hexane as the first fraction, which contained several ortho-substituted PCBs, *p*,*p*′-DDE, and HCB. The second fraction eluted with 30 mL of 50% DCM in hexane contained PCDD/DFs and dioxin-like PCBs, which was purged under a gentle stream of nitrogen to dryness and passed through a silica gel impregnated activated carbon column (0.5 g) to further separate mono- and di-*ortho*-PCBs from non-*ortho*-PCBs and PCDD/DFs. The first fraction, which was eluted with 25 mL of 25% dichloromethane in hexane, contained mono- and di-*ortho*-PCBs. The second fraction eluted with 250 mL of toluene contained non-*ortho*-PCBs and PCDD/DFs. Sample extracts were analyzed by a high-resolution gas chromatograph interfaced with a high-resolution mass spectrometer (HRGC-HRMS). Total PCBs, *p*,*p*′-DDE, and HCB were determined by injecting an aliquot of the corresponding fraction into a gas chromatograph equipped with an electron capture detector as described elsewhere (*18*, *19*).

Identification and Quantification. Identification and quantification of 2,3,7,8-substituted congeners of PCDD/DFs and dioxin-like PCBs were performed using an HRGC (Hewlett-Packard 6890 series) coupled with an HRMS (Mi-

TABLE 2. Concentrations of 2,3,7,8-Substituted PCDDs and PCDFs (Picograms per Gram, Wet Weight) in Bald Eagle Tissues

	sample[a]																			
	373			377		374	378			380	379		381			382		84	94	96	113
	GB	M	L	M	K	L	L	F	M	F	M	L	K	L	M	L	M	P	P	P	P
fat (%)	1.6	1.6	5.7	1.3	5.3	6.4	6.7	75.2	1.3	82	1.87	6.64	3.87	6.45	1.54	5.92	1.87	0.46	0.66	0.55	0.77
2378-D	100	340	370	67	430	1.3	19	1.5	2.1	17	4.7	9.6	6.6	9.3	1.1	7.5	4.1	0.16	0.07	0.64	0.06
12378-D	200	540	730	73	510	3.0	21	2.5	2.2	52	8.6	12	16	27	2.8	27	9.1	0.60	0.45	0.59	0.20
123478-D	54	68	160	4.8	59	0.1	2.9	0.4	0.3	20	1.8	2.5	5.6	9.4	0.8	4.3	1.3	0.28	0.34	0.15	0.09
123678-D	220	240	540	37	300	2.3	14	1.5	1.3	50	6.0	8.4	11	23	2.0	18	6.5	0.48	0.34	0.42	0.14
123789-D	1.8	1.2	3.0	0.5	3.9	0.1	0.1	0.03	0.02	3.6	0.1	0.1	0.3	0.1	0.02	0.2	0.1	0.06	0.01	<0.01	0.01
1234678-D	19	4.7	35	2.7	36	0.6	5.3	0.7	0.3	46	0.5	1.1	2.8	1.8	0.2	2.5	0.4	0.22	1.8	0.27	0.45
OCDD	73	130	980	19	92	2.1	6.3	2.0	1.4	24	2.3	6.9	8.4	18	3.3	6.7	2.1	0.71	19	1.1	0.45
2378-F	25	29	38	1.2	13	1.2	30	7.4	2.3	37	1.0	0.1	4.9	5.3	0.4	4.7	2.0	0.52	0.51	1.0	0.18
12378-F	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.2	4.2	<0.01	13	<0.01	10	0.6	<0.01	<0.01	<0.01	<0.01	0.15	0.13
23478-F	290	560	960	53	470	2.0	15	1.8	1.6	10	4.2	5.0	19	36	2.7	14	8.7	0.29	0.72	0.36	0.12
123478-F	130	260	270	41	470	4.3	41	2.9	4.0	20	12	45	23	56	4.5	13	7.2	0.36	23	0.61	0.13
123678-F	53	63	77	9.1	45	1.1	2.9	0.2	0.3	4.3	1.7	8.6	3.3	5.6	0.6	2.3	1.0	0.14	0.67	0.11	0.09
234678-F	81	99	110	10	55	1.0	3.2	0.2	0.4	3.4	2.0	6.4	5.6	3.6	0.7	2.0	1.1	0.22	0.31	0.14	0.06
123789-F	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.0	<0.01	<0.01	0.9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.09	<0.01
1234678-F	98	190	140	13	45	1.8	5.5	0.4	0.6	5.4	3.3	1.7	13	19	2.0	2.7	1.8	0.16	0.94	0.12	0.09
1234789-F	32	66	44	3.8	25	1.1	2.4	0.2	0.3	2.6	1.7	0.6	14	21	1.9	0.9	0.6	0.07	0.36	0.09	0.09
OCDF	56	120	88	14	36	1.2	5.6	0.4	0.6	3.3	3.3	1.5	18	27	3.4	2.0	1.4	0.13	0.21	0.19	0.06
ΣPCDDs	670	1300	2800	210	1430	10	69	8.6	7.6	210	24	40	50	89	10	67	24	2.5	22	3.2	1.4
ΣPCDFs	770	1400	1700	150	1200	14	110	14	10	92	29	80	102	180	17	42	24	1.9	27	2.9	0.9
PCDDs+DFs	1400	2700	4500	349	2600	23	180	22	18	310	53	120	150	270	27	110	47	4.4	49	6.1	2.3

[a] GB, M, L, K, F, and P indicate gall bladder, muscle, liver, kidney, fat, and plasma, respectively. Values have been rounded.

cromass Autospec-Ultima). The HRMS was operated in an electron impact, selected ion monitoring mode at a resolution of $R > 10000$ (10% valley). Separation was achieved using a DB-5 (J&W Scientific; 0.25 mm i.d. × 60 m length) and a DB-17 column (J&W Scientific; 0.25 mm i.d. × 60 m length). Details of the oven temperature program are given elsewhere (*15*). Prior to injection, ^{13}C-labeled 1234-TeCDD and 123789-HxCDD were added as injection recovery standards. Mean (range) recoveries of spiked internal standards through the whole analytical procedure were 89% (82−106%). PCDD/DF concentrations are presented as picograms per gram on a wet weight basis, whereas the concentrations of total PCBs, dioxin-like PCBs, *p*,*p*′-DDE, and HCB are reported as nanograms per gram on a wet weight basis. TEQ concentrations are expressed as picograms of TEQ per gram on a wet weight basis unless otherwise specified. Concentrations of dioxin-like PCBs refer to the sum of four non-, eight mono-, and two di-ortho-substituted PCB congeners. PCB congeners are represented by the IUPAC numbers. Concentrations have been rounded to two significant digits.

Results and Discussion

PCDD/DF Concentrations. Concentrations of 17 PCDD/DFs were greater in liver (23−4500 pg/g, wet wt) than in kidney (150−2600 pg/g, wet wt), muscle (18−2700 pg/g, wet wt), gall bladder (1400 pg/g, wet wt), fat (22−310 pg/g, wet wt), or blood plasma (2.3−49 pg/g, wet wt) (Table 2). When the values were expressed on a lipid weight basis, muscle tissue contained greater concentrations of PCDDs/DFs followed in decreasing order by gall bladder, kidney, and fat. The greatest concentrations of PCDDs and PCDFs were found in the tissues of birds that died of peritonitis and bacterial infection. These birds were collected from Cecil Bay and Mackinac Island. The least concentration of PCDD/DFs was found in an adult male bald eagle from Ironwood, MI. The least concentration in liver was ∼200 times less than the greatest concentration. The wide variation in concentrations of PCDD/DFs in livers suggests exposure to localized sources of contamination. A few studies have measured concentrations of PCDDs/DFs in livers of bald eagles from British Columbia, Canada (*4*−*7*). Concentrations of PCDD/DFs measured in livers of bald eagles from the Upper Peninsula of Michigan were similar to those reported for eagles collected near a kraft pulp mill in British Columbia. Similarly, concentrations of PCDD/DFs in blood plasma of bald eagles from Michigan were similar to those reported for eagle plasma from British Columbia except one sample, which had a very high concentration of 49 pg/g, wet wt (*7*).

The limited data suggest that concentrations of PCDD/DFs in livers of juvenile bald eagles (mean = 180 pg/g, wet wt) were less than in adults (mean = 1600 pg/g, wet wt) (Table 2). Livers of adult male bald eagles (mean = 2300 pg/g, wet wt) contained greater concentrations of PCDD/DFs than did those of adult female bald eagle (110 pg/g, wet wt). Bald eagles from Cecil Bay and Mackinac contained greater concentrations of PCDD/DFs than those from Paint River, Fence River, Kallio, and Menominee.

Ratios of concentrations of PCDDs to PCDFs varied among tissues and individuals. The ratios of concentrations of PCDDs to PCDFs in livers ranged from 0.48 to 1.6. Even within an individual adult male eagle, the concentration of PCDDs in liver was 1.6-fold greater than that of PCDFs, whereas PCDF concentrations were greater than those of PCDDs in gall bladder and muscle. This suggests tissue-specific distribution of PCDD/DF congeners. Relative distributions of PCDD/DF congeners among liver, muscle, and gall bladder from an individual adult male eagle (sample 373; Table 1) are shown (Figure 2). OCDD, 23478-PeCDF, 12378-PeCDD, 123678-HxCDD, and 123478-HxCDF were the predominant congeners in livers (Table 2). OCDD was relatively less prevalent

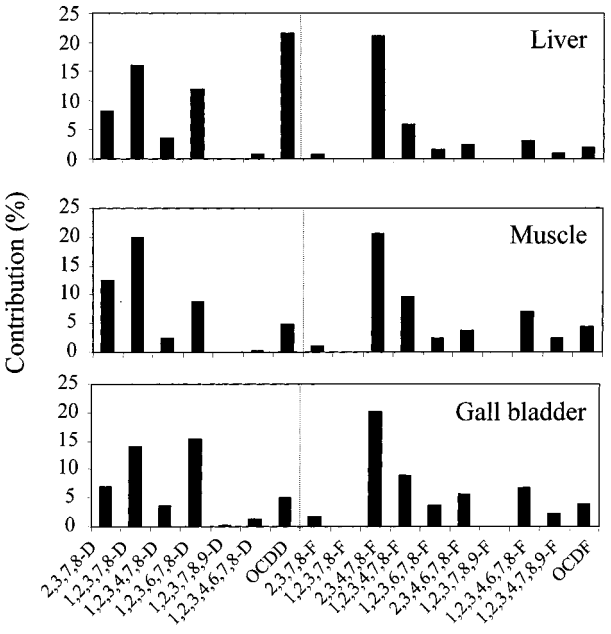

FIGURE 2. Relative contribution (percent) of PCDD/DF congeners to total PCDD/DF concentrations in liver, muscle, and gall bladder of an adult male bald eagle.

in other tissues except blood plasma. Although bile is synthesized in the liver and stored in the gall bladder, OCDD, which was prevalent in the liver, was less abundant in the gall bladder. This suggests slower elimination of OCDD from the liver. In the kidney, 12378-PeCDD was the predominant congener followed in decreasing order by 2378-TCDD, 123678-HxCDD, 123478-HxCDF, and 23478-PeCDF (Table 2). In the gall bladder, 23478-PeCDF predominated followed by 12378-PeCDD, 123678-HxCDD, 2378-TCDD, and 123478-HxCDF (Table 2). The order of PCDD/DF congener concentrations in fat was 2378-TCDF > 123478-HxCDF > 12378-PeCDD > 123678-HxCDD > 1234678-HpCDD (Table 2). Similar to that in liver, OCDD, 12378-PeCDD, 123678-HxCDD, and 123678-HxCDF were the prevalent congeners in blood plasma (Table 2). The relatively great proportion of OCDD in blood plasma has been suggested to be due to binding of this congener to serum proteins (*7*). Fishes collected from Michigan waters of the Great Lakes contained a greater proportion of OCDD and OCDF than the other dioxin homologues (*20*). The most toxic congener, 2378-TCDD, was found in all of the samples at detectable concentrations. In particular, birds that died of peritonitis contained the greatest concentrations of TCDD of 370 and 430 pg/g, wet wt, in liver and kidney, respectively. These values are similar to those reported in the livers of dead bald eagles collected near a kraft pulp mill in British Columbia (*4*). Elevated concentrations of PCDDs and PCDFs observed in two birds that died of peritonitis suggest that these compounds may have played a role in the development of disease. Even if the observed concentrations did not have acute effects, chronic exposures can have significant effects on endocrine and immunological systems (*21*).

Overall, PCDD congeners 12378-PeCDD and 123678-HxCDD and PCDF congeners 23478-PeCDF and 123478-HxCDF were the most predominant congeners in the tissues of bald eagles. This is similar to the pattern observed in eggs of herring gulls and double-crested cormorants collected from the Great Lakes shores of Michigan (*22*). The predominance of PeCDD/DF and HxCDD/DF in bald eagle tissues suggests chlorophenol-related sources originating from kraft pulp milling of contaminated wood chips (*5*). This is further supported by the presence of 2378-TCDD and 2378-TCDF

TABLE 3. Concentrations of Dioxin-like PCBs, Total PCBs, *p,p′*-DDE, and HCB (Nanograms per Gram, Wet Weight) in Bald Eagle Tissues

	sample[a]																			
	373			377		374	378			380	379		381			382		84	94	96	113
	GB	M	L	M	K	L	L	F	M	F	M	L	K	L	M	L	M	P	P	P	P
fat (%)	1.6	1.6	5.7	1.3	5.3	6.4	6.7	75.2	1.3	82.2	1.87	6.64	3.87	6.45	1.54	5.92	1.87	0.46	0.66	0.55	0.77
non-*ortho*-PCBs																					
344′5-TCB (81)[b]	1.6	4.9	4.9	0.6	2.7	0.01	0.03	0.6	0.04	0.1	0.1	0.1	0.2	0.6	0.1	0.1	0.03	0.002	0.0008	0.0008	0.0003
33′44′-TCB (77)	22	24	30	12	56	0.7	1.0	18	1.4	5.7	1.6	1.2	7.1	18	2.1	3.9	2.1	0.15	0.017	0.036	0.015
33′44′5-PCB (126)	28	22	33	7.9	38	0.4	0.4	7.9	0.5	3.7	2.2	1.7	4.0	11	1.2	1.3	0.8	0.025	0.023	0.040	0.006
33′44′55′-HxCB (169)	7.2	13	19	1.4	7.2	0.2	0.05	1.2	0.1	1.0	0.5	0.3	1.5	4.5	0.4	0.7	0.4	0.003	0.007	0.009	0.0012
mono-*ortho*-PCBs																					
233′44′-PCB (105)	3900	1900	4000	970	4200	26	35	740	51	260	180	130	330	170	110	130	80	1.8	0.76	1.8	0.23
2344′5-PCB (114)	640	1300	1500	150	600	5.3	4.3	100	6.5	28	25	16	49	26	19	11	6.6	0.14	0.043	0.13	0.02
23′44′5-PCB (118)	6200	2100	3700	1500	6100	110	92	1500	130	710	490	400	1700	910	490	410	240	4.6	3.6	4.6	0.89
2′344′5-PCB (123)	480	890	1200	130	510	3.4	4.4	110	6.8	27	21	14	31	17	12	16	9.4	0.17	0.09	0.17	0.03
233′44′5-HxCB (156)	1400	1500	1700	360	1500	47	14	320	22	170	110	76	330	170	99	74	45	0.52	0.52	0.83	0.19
233′44′5′-HxCB (157)	290	670	760	96	400	7.1	3.2	78	5.1	43	23	15	46	25	15	16	10	0.16	0.18	0.23	0.05
23′44′55′-HxCB (167)	850	1200	1300	210	890	40	8.2	220	14	120	72	48	280	150	86	40	25	0.34	0.44	0.56	0.13
233′44′55′-HpCB (189)	180	460	510	51	200	3.5	0.6	39	1.0	18	16	7.0	40	21	15	5.3	2.7	0.05	0.06	0.09	0.025
di-*ortho*-PCBs																					
22′33′44′5-HpCB (170)	2700	21000	44000	820	3500	200	37	370	54	450	290	200	810	440	230	200	120	1.2	1.4	0.40	2.6
22′344′55′-HpCB (180)	5400	26000	100000	1500	6500	680	91	1900	140	1200	670	540	3300	920	690	510	290	3.5	4.5	1.3	9.6
total PCBs[c]	48000	63000	280000	17000	52000	1900	450	17000	1100	8100	4200	2300	11000	16000	2000	3300	2000	67	47	64	46
p,p′-DDE	2800	690	17000	750	2900	58	45	2300	170	1600	530	230	290	3500	220	620	300	8.0	11	15	5.7
HCB	18	20	120	23	45	4.1	1.6	30	1.7	29	2.2	1.2	28	40	2.7	16	6.5	0.17	<0.01	0.48	0.13

[a] GB, M, L, K, F, and P represent gall bladder, muscle, liver, kidney, fat, and plasma, respectively. Values have been rounded. [b] Numbers in parentheses indicate IUPAC numbers. [c] Total PCBs refers to the sum of all congeners.

TABLE 4. TCDD Toxic Equivalents (Picograms of TEQ per Gram, Wet Weight) in Bald Eagle Tissues

	sample[a]																			
	373			377		374	378			380	379		381			382		84	94	96	113
	GB	M	L	M	K	L	L	F	M	F	M	L	K	L	M	L	M	P	P	P	P
fat (%)	1.6	1.6	5.7	1.3	5.3	6.4	6.7	75.2	1.3	82.2	1.87	6.64	3.87	6.45	1.54	5.92	1.87	0.46	0.66	0.55	0.77
PCDDs	310	890	1100	140	950	4.4	41	4.0	4.4	71	14	22	23	37	4.0	35	13	0.78	0.54	1.2	0.28
PCDFs	340	630	1000	61	540	3.9	50	10	4.4	51	6.8	12	27	49	3.8	21	12	0.88	3.6	1.5	0.33
non-*ortho*-PCBs	4100	3900	5400	1400	6900	77	91	1800	130	670	310	230	780	2100	230	330	190	10	3.2	5.9	1.3
mono-*ortho*-PCBs	700	580	860	180	750	10	6.7	140	10	59	40	28	96	50	30	28	17	0.31	0.19	0.35	0.06
total TEQs	5500	6000	8400	1800	9100	95	190	2000	150	850	370	290	930	2200	270	410	230	12	7.6	9.0	2.0

[a] GB, M, L, K, F, and P represent gall bladder, muscle, liver, kidney, fat, and plasma, respectively. Values have been rounded.

in tissue samples. The predominance of 23478-PeCDF has been attributed to exposure to technical PCB mixtures, which contain this isomer as a major impurity (*5*). Concentrations of total PCBs in the bald eagle samples were also relatively great, which supports the hypothesis that technical PCB mixtures may have been a source of PCDD/DFs in bald eagles.

The ratios of concentrations of TCDD to TCDF was 0.65−10 in livers, 0.93−57 in muscle, 1.35−34 in kidney, 3.97 in gall bladder and and 0.20−0.45 in fat tissue. It is interesting to note the shift in TCDD/TCDF ratios as a function of TCDD concentrations. In eagle tissues containing the greatest concentrations of TCDD (67−430 pg/g in various tissues), concentrations of TCDF were less. That is, the mean TCDD/TCDF ratio for these tissues was 23 (range = 4−57). Alternatively, the mean TCDD/TCDF ratio was ≤1 (range = 0.2−4.7) for other individuals containing lesser concentrations of TCDD. The shift in the ratios may suggest induction of hepatic cytochrome P450 enzymes (*23*, *24*) in eagles exposed to elevated concentrations of TCDD, and consequently TCDF was metabolized. TCDF is relatively more rapidly excreted from the body than TCDD (*23*). The shift in TCDD and TCDF ratios as a function of total TCDD concentrations has previously been observed in bald eagles (*4*).

PCB Concentrations. Concentrations of total PCBs in livers of bald eagles ranged from 450 to 280,000 ng/g, wet wt (Table 3). Two individuals with the greatest concentrations of PCDD/DFs also contained the greatest concentrations of total PCBs. The *p*,*p*′-DDE concentration in the liver of one of the diseased individuals was 17,000 ng/g, wet wt (Table 3). The concentration of 280,000 ng PCBs/g, wet wt, is the greatest value reported for a bald eagle liver so far. The greatest concentration of total PCBs recorded in this study was 3−4-fold greater than those reported for bald eagle livers collected near a kraft pulp mill in British Columbia (*4*). Concentrations of total PCBs in blood plasma ranged from 46 to 67 ng/g, wet wt (Table 3). These concentrations are similar to those reported for plasma of bald eagles from British Columbia (*7*) but less than those found in bald eagle plasma from Lake Erie (*8*).

The total concentrations of 14 dioxin-like PCB congeners in bald eagle tissues (all of the tissues) collectively accounted for 50% of the total PCB concentrations. Among non-*ortho*-PCBs, IUPAC 77 was the most predominant isomer in 13 of the 17 tissue samples analyzed followed by IUPAC 126 > 169 > 81 (Table 3). In the liver and gall bladder of the individual that contained the greatest PCB concentration, IUPAC 126 was the most predominant congener followed in the order of 77 > 169 > 81. This provides additional evidence of induction of cytochrome P450 enzymes in eagles exposed to elevated concentrations of Ah receptor agonists such as PCBs, PCDDs, and PCDFs. Induction of cytochrome P450 enzymes may have resulted in the metabolism of lower chlorinated non-ortho coplanar PCB congener 77, which subsequently increased the relative abundance of congener 126 in livers. Increases in PCB126 to PCB77 ratios as a function of an increase in total PCB concentrations have been reported earlier (*24*, *25*). Among mono-*ortho*-PCBs, IUPAC 118 was the most prevalent congener in all of the tissues followed by 105 > 156 > 167 > 114 > 123 > 157 > 189. A similar trend in PCB congener distribution was observed in several species of water birds (*26*). Di-*ortho*-PCBs 170 and 180 are the most abundant congeners found in eagle tissues. A concentration of PCB congener 180 was as great as 100,000 ng/g, wet wt, in the liver of an adult male eagle collected from Cecil Bay, which was 35% of the total PCB concentrations (Table 3). Elevated exposures to total PCBs, PCDDs, and PCDFs in this individual bird may have resulted in the induction of cytochrome P450 enzymes, causing the metabolism of lower chlorinated congeners and relative enrichment of higher chlorinated congeners such as PCB180.

Concentrations of 14 dioxin-like PCBs in the blood plasma of eagles ranged from 10 to 14 ng/g, wet wt (Table 3). This was 15−30% of the total PCB concentrations. Concentrations of the non-ortho congener 77 were greater than or similar to that of congener 126 in plasma. Congeners 169 and 81 were present at small concentrations. Similar to that found in other tissues, mono-*ortho*-PCB congener 118 was the most prevalent followed in decreasing order by 105 > 156 > 167 > 114 ≥ 157 > 123 ≥ 189 in all of the plasma samples analyzed. The pattern of PCB congener distribution in the plasma of bald eagles was similar to that reported for the plasma of bald eagles from British Columbia (*7*).

TEQs. Each of the 17 2,3,7,8-substituted PCDD/DF congeners and 12 dioxin-like PCBs (two di-*ortho*-PCBs were not included because WHO TEFs for these two congeners were not presently available) has been assigned a TEF based on their toxic potency relative to that of 2378-TCDD, which is assigned a TEF of 1 (*14*). Multiplication of the concentrations of PCDD/DF congeners and dioxin-like PCBs by their TEF provides TEQ concentrations for a mixture of toxic congeners. Although several TEF schemes have been proposed, the WHO TEFs for birds were applied for the estimation of TEQs (*14*). Concentrations of TEQs in bald eagle livers ranged from 95 to 8400 pg/g, wet wt, followed by kidney (930−9100 pg/g, wet wt), muscle (150−6000 pg/g, wet wt), gall bladder (5500 pg/g, wet wt), fat (850−2000 pg/g, wet wt), and blood plasma (2−12 pg/g, wet wt) (Table 4). Livers and kidneys of bald eagles that died of peritonitis contained the greatest TEQ concentrations of 8400 and 9100 pg/g, wet wt, respectively. These values are greater than those reported for livers of dead bald eagles collected near a kraft pulp mill in British Columbia (*4*). Concentrations of TEQs in bald eagle tissues fall within the range of toxic threshold values reported for chicken, pheasant, or Caspian tern eggs (*6*, *27*). A lowest-observed-effect level (LOEL) of 25 ng of TEQ/g in liver on a lipid weight basis has been suggested for CYP1A induction and 50% reduction of plasma thyroxine levels in common tern chicks (*28*). On a lipid weight basis, concentrations of TEQs in livers of all the birds analyzed in this study were

TABLE 5. Estimated Burdens of Organochlorines in Body Fat and Biliary Excretion Rates in Bald Eagles

compd	mean concn in body fat[a] (μg/g, lipid wt)	amount (burden) in body fat (μg)	concn in bile/gall bladder (μg/g, lipid wt)	amount (burden) in bile (μg)	biliary excretion rate (% per day)
PCDDs	0.066	43.6	0.042	0.0067	0.015
PCDFs	0.059	38.7	0.048	0.0077	0.020
PCBs	4430	2920000	3000	480	0.016
p,p′-DDE	170	113000	175	28	0.025
HCB	1.7	1100	1.13	0.18	0.016

[a] Mean of muscle and liver concentrations (lipid weight basis).

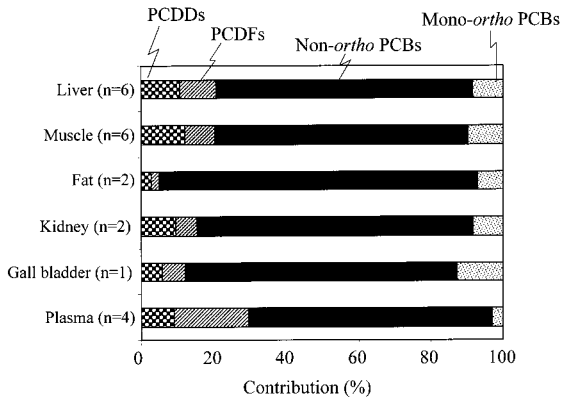

FIGURE 3. Relative contribution (percent) of PCDDs, PCDFs, non-*ortho*-PCBs, and mono-*ortho*-PCBs to total TEQs in tissues of bald eagles.

>1000 pg/g. Two of the adult male bald eagles contained TEQ concentrations >25 ng/g, lipid weight, a dose suggested to reduce blood thyroxine levels of common terns (*28*). A threshold reference value of 2300 pg of TEQ/g, wet wt, in eggs has been proposed for American kestrels (*29*). The concentrations of TEQs in livers of all the bald eagles analyzed, except one, were greater than the toxic reference values reported for American kestrels. The concentrations of TEQs in tissues were greater than the no-observed adverse effect level (NOEL) of 100 pg/g, wet wt, proposed for bald eagle eggs (*6*).

Non-*ortho*-PCBs contributed the greatest proportion of TEQs in all of the tissue samples. The contribution of non-*ortho*-PCBs to total TEQs in eagle tissues ranged from 48 to 94% in liver, from 65 to 87% in muscle, from 75 to 84% in kidney, from 79 to 92% in fat, and from 52 to 84% in plasma and was 75% in gall bladder (Figure 3). On average, non-*ortho*-PCBs contributed 68−88% of the TEQs of total PCBs. Contribution of PCDDs to TEQs ranged from 1.7 to 22% in liver, from 1.5 to 15% in muscle, from 2.5 to 10% in kidney, from 0.2 to 8.3% in fat, and from 6 to 14% in plasma and was 5.7% in gall bladder. Contribution of PCDFs to TEQs ranged from 2.2 to 27% in liver, from 1.4 to 11% in muscle, from 2.9 to 5.9% in kidney, from 0.5 to 6.0% in fat, and from 7 to 48% in plasma and was 6.2% in gall bladder. PCDDs and PCDFs collectively accounted for 17% of the total TEQs in tissues.

Biliary Excretion of Organochlorines in Bald Eagles. The gall bladder analyzed in this study was filled with bile. Assuming that organochlorine concentrations measured in the gall bladder would reflect that in bile, biliary excretion of organochlorines could be estimated. The biliary excretion rate can be calculated as

$$\text{biliary excretion rate (\% per day)} = [\text{amount in bile/gall bladder } (\mu\text{g}) \div \text{amount in body fat } (\mu\text{g})] \times 100$$

For this estimation, data for muscle, liver, and gall bladder from the same individual were used (sample 373; see Table 1). The excretion rate is calculated on the basis of the amount (burden) of contaminants in body fat and the amount (burden) in bile/gall bladder. It was assumed that the fat content of bald eagle was 20% and bile secretion rate was 10 g per day (*30*). The body weight of the eagle was 3.3 kg, which would correspond to 660 g of fat. The lipid content of bile/gall bladder was 1.6% (Table 2), which would correspond to 0.16 g of daily fat excretion through bile. Because target organochlorines accumulate in lipids, lipid normalized concentrations in liver and muscle, on average, provided body burden of organochlorines. Burdens in body fat and bile were calculated as

$$\text{organochlorine burden in body fat } (\mu\text{g}) = \text{mean concentration in muscle and liver } (\mu\text{g/g on a lipid weight basis}) \times 660 \text{ g (mass of fat)}$$

$$\text{organochlorine burden in bile } (\mu\text{g}) = \text{concentrations in bile/gall bladder } (\mu\text{g/g, lipid wt}) \times 0.16 \text{ g (mass of fat)}$$

Excretion rates of PCDDs, PCDFs, PCBs, *p,p′*-DDE, and HCB ranged from 0.015 to 0.025% per day (Table 5). These values are an order of magnitude lower than those reported for TCDD in humans (*31*) but comparable to those reported for herring gulls (*23*). Although it is known that the excretion rates of PCBs, PCDDs, and PCDFs are congener-specific, due to the small sample size, such analysis was not performed here. Nevertheless, this study provides a preliminary estimate of biliary excretion in bald eagles for the first time. Biliary excretion accounts for only a small proportion of the elimination of xenobiotics from the body (*32*). Bile can undergo extensive resorption within the intestinal tract during enterohepatic circulation.

These results suggest that concentrations of PCDDs, PCDFs, and PCBs from the Upper Peninsula of Michigan were similar to those in birds collected near a kraft pulp mill facility in British Columbia. Non-ortho coplanar PCBs were the major contributors to dioxin-like toxicity. Biliary excretion rates of PCDDs, PCDFs, and PCBs in bald eagles were 0.015−0.025% per day.

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