BIOACCUMULATION AND TOXICOKINETICS OF 42 POLYCHLORINATED BIPHENYL CONGENERS IN AMERICAN KESTRELS (FALCO SPARVERIUS)

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Abstract—The bioaccumulation and toxicokinetics of 42 polychlorinated biphenyls (PCBs) was determined in male American kestrels exposed to an Aroclor®-contaminated diet for 120 d followed by a 348-d depuration period. The birds were housed under ambient outdoor temperatures to permit normal fluctuations in body weight during the study. Whole body PCB clearance, plasma/fat distribution coefficients, and plasma PCB clearance constants were determined for individual PCBs to calibrate a two-compartment rate constant model in order to describe PCB elimination in the birds. Plasma/fat partition coefficients (Kp) averaged 0.0060 ± 0.0001 for all congeners of study, were not dependent on chemical hydrophobicity, and did not change in summer versus winter sacrificed animals. Plasma clearance constants (kpc) for PCBs were observed to be dependent on both chlorine substitution patterns and congenenter hydrophobicity. Polychlorinated biphenyl congeners categorized as readily cleared congeners contained vicinal meta–para hydrogen substituents on at least one phenyl ring, while slowly cleared congeners were chlorine hindered at these positions. A general equation was derived to predict plasma clearance constants for all tri- to octachlorobiphenyls based on the presence of an open meta–para site on one of the phenyl rings and from the n-octanol–water partition coefficient of the chemical. The equation was validated by comparing predicted versus measured relative biomagnification factors of PCBs determined in birds at the end of the dosing period. The two-compartment model calibrated for PCB elimination in American kestrels may be used to describe PCB toxicokinetics in wild birds provided that seasonal fluctuations in the fat content of the modeled population is known.

Keywords—Elimination kinetics Raptor Exposure dynamics Metabolic biotransformation

INTRODUCTION

Birds have often been used as biomonitorst of toxicity and exposure to pollutants in the environment [1–3]. Among the persistent polyhalogenated aromatic hydrocarbons (PHAHS) typically monitored, polychlorinated biphenyls continue to comprise a major component of measured environmental residues [3,4]. Models describing the bioaccumulation of PCBs and other organochlorines in birds have been described only to a limited extent [5–7]. Such models are necessary for the interpretation of biomonitoring trends, especially when the species of study may be exposed to multiple food sources and/or accumulate contaminants at different locations due to migration habits. Few experimental studies have documented key toxicokinetic parameters of PCBs such as contaminant elimination rates in nondomesticated species [8,9]. In addition, much of the earlier work that had documented PCB clearance in birds such as chickens [10] and pheasant [11] had employed low-resolution packed column chromatography techniques that precluded the measurement of individual PCB congeners.

Congener patterns of PCBs in several bird species have been found to be remarkably similar despite differences in feeding habits and trophic status [12,13]. The more commonly encountered congeners in environmental samples exhibit a range of physical–chemical properties (i.e. two order of magnitude differences in solubilities [14]) and structural features (degree chlorination and chlorine substitution patterns) that lead to differences in bioaccumulation potentials within birds and their prey items. Studies that examined congener patterns in birds relative to their prey [15,16] or to the original congener composition in Aroclor source mixtures [17,18] indicate that PCBs can be broadly classified into persistent or readily cleared congener groups on the basis of presence of vicinal hydrogen substituents at meta–para sites on at least one of the phenyl rings. Such a classification has been confirmed in controlled laboratory exposures using chickens [19], pigeons [20], and common eiders [21]; however, these latter studies were not carried out for sufficient time periods to characterize differences in individual congener behaviors within a given persistence class.

In this study, elimination rate constants, plasma/fat distribution coefficients, and bioaccumulation potentials of 42 PCB congeners were determined in male American kestrels housed under ambient outdoor temperature conditions. The animals were exposed to an Aroclor mixture (1248:1254:1260 1:1:1 by wt) added to their diet for 120 d and subsequently allowed to depurate by feeding a control diet for an additional 348 d. Trends associated with the elimination of individual PCBs were used to test the structure activity rules regarding persistence of PCBs in birds and to provide a more comprehensive data set for describing elimination trends of PCBs within the readily cleared and persistent congener groups.
Toxicokinetic parameters were derived to calibrate a two-compartment rate constant model as described in Clark et al. [5] to describe PCB elimination in kestrels during the depuration period of the study. The model consists of a peripheral storage compartment of fat and a central compartment of blood plasma. The fat compartment is considered inert with respect to elimination processes and consists of the sum of all tissue lipids (with the exception of blood plasma lipids) of the animal. Whole body elimination processes are assumed to occur in, or are rate limited by, the distribution of contaminant in blood plasma, and the volume of the central compartment is set equal to the volume of plasma in the animal. It should be noted that this approach differs from traditional compartment models that partition the animal into slowly perfused (peripheral compartment, which may include adipose and skin tissues) and richly perfused (central compartment, which may include blood plasma, liver, muscle, and brain tissues) tissue pools. The simplified approach as taken in Clark et al. [5] and in the present study is appropriate for highly hydrophobic chemicals such as PCBs, which are distributed primarily within the lipid component of tissues [22] and when intertissue distribution kinetics within the animal generally exceed the rate of whole body elimination for the chemical of study [20,22].

The chemical flux (ng/d) in the fat compartment is described by

\[ V_f \cdot \frac{dC_f}{dt} = C_f \cdot V_f \cdot k_{pf} - C_f \cdot V_f \cdot k_{fp} \]  

where \( C_f \) is the chemical concentration (ng/g) in whole body tissue lipids, \( V_f \) refers to the total volume (ml) of lipids in animal tissues, and \( \rho_f \) is the density (g/ml) of lipids. Similarly, the total blood plasma volume (ml) and chemical concentrations (ng/ml) in blood plasma are designated in Equation 1 by \( V_p \) and \( C_p \), while rate constants (per day) describing chemical flux from blood plasma to fat and from fat to blood plasma are designated by \( k_{pf} \) and \( k_{fp} \), respectively. During the elimination period, the chemical flux (ng/d) in blood plasma is given by

\[ V_p \cdot \frac{dC_p}{dt} = C_p \cdot V_p \cdot k_{pf} - C_p \cdot V_p \cdot k_{fp} \]  

where the rate constant (per day), designated as \( k_{pf} \), integrates chemical elimination that results from contaminant losses to air via lungs, losses of parent compound to feces via organism/fecal partitioning, and metabolic biotransformation in the liver. Equation 2 was further simplified by Clark et al. [5] by assuming that intertissue distribution kinetics are rapid relative to whole body elimination kinetics (\( k_{pf} V_f C_f \) and \( k_{fp} V_p C_p \) \( \gg \gg \gg \) \( k_{pf} V_f C_p \) and \( k_{fp} V_p C_f \)) and \( k_{fp} V_f C_f \) and \( k_{pf} V_p C_p \) exceed the rate of tissue growth and weight loss during seasonal changes in fat compartment size. Under these conditions it can be assumed that plasma and fat PCB concentrations maintain steady state during the elimination period and can be related to each other using a constant plasma/fat distribution coefficient:

\[ K_{pf} = \frac{C_p}{\rho_f \cdot \rho_f} = \frac{k_{pf}}{k_{fp} \cdot V_p} \]  

Combining Equations 2 and 3 and yields

\[ V_p \cdot \frac{dC_p}{dt} = -C_p \cdot V_p \cdot k_{pf} \cdot V_p \cdot k_{pc} \]  

Since \( V_p \) is species and body-weight dependent, the term \( k_{pc} V_p \) is combined into a single term to define the plasma clearance constant:

\[ k_{pc} = k_{pc} \cdot V_p \cdot BW^{-1} \]  

where \( k_{pc} \) is the plasma clearance constant (ml/g/d) and BW is the body weight (g) of the organism. Incorporation of \( V_p \) into the rate constant expression facilitates comparison of toxicokinetic parameters associated with chemical clearance in other species. For this study, the ratio of \( V_p / BW \) was assumed to be constant at 0.06 ml/g [23,24]. Combining Equations 4 and 5 yields a generalized expression for modeling PCB elimination kinetics in the dosed birds:

\[ V_p \cdot \frac{dC_p}{dt} = -C_p \cdot V_p \cdot k_{pf} \cdot BW \cdot k_{pc} \]  

Under circumstances of changing fat volume with time, as may be encountered in wild populations of birds exposed to seasonal changes in temperature or during migration or reproductivity output [25], \( k_{pc} \) may be determined by a mass balance approach whereby the mass of chemical eliminated from the animal over the depuration portion of the study is normalized to the area under the plasma PCB concentration-time curve (AUC) [5]:

\[ k_{pc} = \frac{X_{b(o)} - X_{b(348)}}{AUC} \cdot 0.06 \]

\[ = \frac{X_{b(o)} - X_{b(348)}}{AUC} \cdot \sum_{i=0}^{n-1} \left( \frac{C_{p(i)} \cdot V_{p(i)} + C_{p(i+1)} \cdot V_{p(i+1)}}{2} \right) \]

where the term \( (X_{b(o)} - X_{b(348)}) \) is the mass (ng) of chemical eliminated by the bird during the elimination period and 0.06 refers to the volume of blood plasma (ml) in the bird normalized to its body weight (g) [23,24].

The objectives of this study were to determine \( k_{pc} \) and \( K_{pf} \) parameters for individual PCB congeners in American kestrels using an elimination study described in the following. By calibrating the previously described toxicokinetic parameters in this species, the model as outlined in Equations 1 to 6 can be extrapolated to wild birds provided that seasonal changes in fat volumes are accounted for in the modeled population.

**Chemicals**

Aroclor 1248 and 1260 was obtained from the U.S. Environmental Protection Agency (Research Triangle Park, NC, USA). Aroclor 1254 was obtained from Supelco (Mississauga, ON, Canada). Seven [U-13 C]-labeled PCBs, IUPAC 28, 52, 118, 126, 138, 153, 180, and 194, were obtained from Cambridge Isotopes (Andover, MA, USA) for use as internal standards. Reported purities for individual compounds were 99%. All solvents used during sample extractions were of pesticide/GC grade.

**Experimental**

The birds utilized in this study were part of a larger investigation to assess reproductive toxicity and multigenerational effects of PCBs in American kestrels [26]. As such, only a subset of experimental birds were sacrificed to calibrate the toxicokinetic model. Male birds were utilized to remove the
confounding effect of egg elimination on calculated toxicokinetic parameters. Seventeen adult male nonbreeding American kestrels were maintained in communal pens under ambient environmental temperatures/photoperiod at the Raptor Centre (McGill University, St. Anne de Bellevue, PQ, Canada). In addition to these birds, seven males, which were used in breeding pairs during the first four months of study, were blood sampled and sacrificed after 148 and 348 d of depuration to supplement the data on nonbreeder males. Both breeders and nonbreeders were given free access to the same diet of dead chicks, which were contaminated with PCBs during March to July for a total exposure period of 120 d. The contaminated food was prepared by injection of 100 μl of an Aroclor mixture (1248:1254:1260) 1:1:1 by weight, dissolved in safﬂower oil, to the brain of dead chicks. Observations on feeding habits of the captive birds indicated preferential feeding on the heads of the dead chicks, and delivery of PCBs into the heads was considered the most consistent means of maintaining constant exposure conditions. The spiking solution was formulated to produce an initial concentration of 0.49 ± 0.02 mg/100 μl sum PCB during March and was subsequently reduced to 0.32 ± 0.03 mg/100 μl oil during April to July feedings. These formulations were intended to maintain an average dose of 5 to 7 μg sum PCB/g bird/d. After the exposure period, birds were maintained on a diet of clean chicks for a further 348 d.

Blood samples (~1 ml) were drawn from the jugular from five randomly captured birds on days 0, 10, 20, 40, 80, 97, and 110. On the last day of dosing, all 17 nonbreeder males were blood sampled to obtain a measure of interindividual variability in PCB exposures. Blood samples were also taken from five randomly captured birds during the depuration period following 20, 41, 71, 92, 148 (three birds), and 348 (10 birds) d after the last dosage. Blood samples were taken from control birds during 0, 20, 60, and 97 d of exposure to determine background PCB concentrations. The heparinized blood samples were centrifuged to separate cellular components, and plasma was stored frozen (~20°C) until chemical analysis. Five male nonbreeder birds were sacrificed on the last day of exposure (day 120), three birds (one nonbreeder male and two breeder males) after 148 d of elimination, and 10 birds (five nonbreeder and five breeder males) after 348 d of elimination. At sacriﬁce, the feathers, feet, intestinal tract, and head were removed, and biopsies of liver, adrenal, kidney, spleen, testis, and lung were taken for histological examination. The total tissue removed for biopsies was less than 0.5 g and was not expected to affect the lipid mass balance in the animal. The remaining carcass was added to a solvent-rinsed stainless-steel food processor for sufﬁcient duration to permit complete homogenization of tissue components. A 25-g aliquot of the homogenate was stored frozen in solvent-cleaned glass jars until chemical analysis. This study was performed with permission of the Canadian Council on Animal Care (Montreal, PQ, Canada).

Analytical

Polychlorinated biphenyls were extracted from plasma using a solid-phase extraction procedure. Plasma (0.3–0.5 ml) was spiked with six 13C-PCB internal standards (IUPAC 28, 52, 118, 153, 180, and 194), deproteinated with methanol (100% plasma mass), vortexed for 1 min, and diluted to 5 ml with distilled water. The sample was loaded onto precleaned, activated C18 solid-phase cartridges (Enviro-18 1 g; Supelco, Mississauga, ON, Canada) and eluted under reduced pressure at 1 ml/min. Analytes were recovered from the extraction column, after drying by vacuum, by elution with 12 ml dichloromethane-hexane (1:1). Cleanup of concentrated extracts was performed by florisil chromatography. Concentrated extracts (~0.5 ml) were loaded onto 1-cm-i.d. × 24-cm glass columns packed with 8 g florisil (1.2% deactivated; BDH Laboratory Supplies, Toronto, ON, Canada) and a 1-cm Na2SO4 cap. The PCBs were eluted from the florisil with 50 ml hexane. The cleaned-up extracts were concentrated to 100 μl and spiked with 13C-CB138 for use as a volume corrector. Blanks were performed as described previously using distilled water. Recoveries of individual internal standards from plasma samples averaged 77 ± 6%.

Carcass homogenates (1–2 g) were ground with Na2SO4 and spiked with the six [13C]-PCB internal standards. The homogenates were packed in glass columns containing 50 ml dichloromethane-hexane (1:1), allowed to stand for 1 h, and eluted with a further 150 ml dichloromethane-hexane at a rate of 1 to 2 ml/min. The extracts were evaporated to 10 ml, a 10% portion of the sample was removed for lipid determination by weighing, and the remaining extracts were cleaned up by gel permeation chromatography [27]. Further cleanup of samples was performed by florisil chromatography as described previously. The extracts were concentrated to 2 ml and spiked with 13C[CB138 as a volume corrector. Blanks were performed as described previously using Na2SO4. Recoveries of individual internal standards averaged 79 ± 7%. Concentrations of individual PCB congeners in plasma and carcass homogenates were corrected for recoveries of the equivalent internal standard isomer. Gas chromatographic analysis was performed on a Hewlett-Packard (Orangeville, ON, Canada) 5890 equipped with an HP-5972 mass selective detector and HP-7673 autosampler. The column was a 30-m DB-5, fused silica capillary column (0.25-μm film thickness; Chromatographic Specialties, Brockville, ON, Canada). Chemicals were identiﬁed by mass spectrometer retention time under selective ion monitoring. Quantitation of individual PCBs was based on the response factors from a secondary standard containing Aroclor 1242:1254:1260 (1:1:1). Details of the secondary standard calibration are provided in [15]. Standards were injected for every ﬁve samples analyzed.

Data analysis

Unless otherwise stated, all reported values refer to the mean ± standard error. Linear regression, analysis of variance (ANOVA), and analysis of covariance was performed using SYSTAT software after testing for normality using normal probability plots [28]. Nonlinear regressions were performed using SYSTAT software. Nonlinear regression ﬁts were performed several times using different initial starting coefﬁcients in order to verify the optimum solution.

RESULTS

Small quantities of PCBs (sum PCB = 10.3 ± 3 ng/ml) were detected in blood plasma of control birds taken during the accumulation portion of the experiment and in blood plasma of birds sampled prior to dosing on day 0. The composition of PCBs in controls consisted primarily of persistent congeners PCB28, 99, 118, 138, 153, and 180. These congeners are typically found in environmental samples and may reﬂect background contamination of the study birds. The levels of PCBs in control plasma were 205-fold lower than treatment birds after 10 d exposure to the contaminated diet and from 100-
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800-fold lower than residues observed in treatment birds at the end of the 348-d elimination period. The exception was for PCB28, for which residue levels in control plasma were 20% of those observed in eliminating birds after the 348-d elimination period. Concentrations of PCB28 in plasma of treatment birds were adjusted by subtracting the maximum levels observed in controls to account for potential accumulation of this chemical prior to dosing.

Trends in plasma PCB concentrations for 12 representative chemicals during the uptake and elimination portions of the study are summarized in Figure 1. Average plasma PCB residues in the sampled birds increased linearly during the first 80 d of the dosing period for all chemicals and became relatively constant during the last three sampling events of the uptake period. However, no attempts were made to quantify plasma PCB uptake rate constants because of complications associated with differences in feeding behavior of individuals (feeding on heads or body of dead chicks) and because of changes in the concentration of PCBs in the dosing oil given to all birds during the uptake period.

Blood plasma and carcass PCB residues in birds used as breeders during the uptake portion of the study were not significantly different (ANOVA; \( p > 0.4 \)) than PCB residues in nonbreeder males sampled at the same time. Therefore, data for breeder and nonbreeder males were pooled in subsequent analyses. Whole body PCB burdens were determined from carcass homogenates of animals sacrificed at the peak of exposure (\( n = 5 \)) and in animals sacrificed after 348 d of depuration (\( n = 10 \)). The average whole body clearance, expressed as the percentage of dose lost over 348 d, for individual PCB congeners is summarized in Table 1. As predicted by the general structure activity relationships describing PCB persistence in birds, whole body clearances were elevated for congeners having the common structural feature of vicinal hydrogen substituents at meta–para sites on at least one of the phenyl rings (having one or two open meta–para sites). From the data of Table 1, it is evident that all PCBs having an open meta–para site were completely eliminated from animals during the depuration period. In contrast, PCB congeners that did not contain an open meta–para site on either phenyl ring (congeners having at least 4,4', 3,3', 5,5', 4,3', 5', or 3,4', 5 chlorine substitution patterns) exhibited variable whole body clearances ranging from 44 to 98% of the average PCB body burden in day 0 elimination birds.

Calibration of the two-compartment rate constant model in American kestrels

Plasma PCB concentrations exhibited declining trends during the depuration period for all congeners studied. As observed with whole body clearance values, PCBs having an open meta–para site on at least one phenyl ring were found to decline in plasma in a log linear manner and were generally not detectable in blood after 148 d of elimination (Fig. 1, upper graphic). In contrast, the persistent PCB congeners were observed to exhibit slower and less linear declining trends in plasma with time (Fig. 1, middle graphic).

Average body weights of kestrels were 111.1 ± 0.9 g over the study period but were variable among individuals and seasonally dependent (Fig. 1, lower graphic). It is of interest to note that the rapid declines associated with plasma PCB concentrations observed for slowly cleared PCBs during August to November of the study were consistent with an increasing trend in the body weights of the same sampled birds (Fig. 1).

Fig. 1. Plasma polychlorinated biphenyls (PCB) trends and seasonal changes in body weight of male American kestrels. Upper graphic presents plasma trends of PCBs containing vicinal hydrogen substituents at meta–para sites on at least one phenyl ring. Each point represents the mean ± standard error of blood concentrations in replicate (\( n = 5 \)) birds. Symbols refer to PCB 52 (■), PCB 64 + 41 + 71 (○), PCB 101 (▲), PCB 141 (◇), PCB 149 (*), PCB 174 (○). Middle graphic presents plasma trends of PCBs which are chlorine hindered at meta–para sites on both phenyl rings. Symbols refer to PCB 28 (■), PCB 66 (○), PCB 118 (▲), PCB 138 (◇), PCB 180 (*), and PCB 194 (○). Solid line indicates the end of dosing and beginning of the 348 d depuration period. Bottom graphic presents trends in male American kestrel body weights during the study duration.
These results suggest that trends in whole body elimination, as determined by a simple analysis of plasma PCB concentration trends, would be confounded by changes in the fat compartment size of birds with time. As such, plasma clearance constants ($k_{pc}$) for PCBs were calculated using Equation 7 and assuming a destructive sampling design as outlined in Hothorn et al. [29].

Plasma clearance constants for individual PCB congeners are summarized in Table 1. Trends in $k_{pc}$ with chemical $K_{ow}$ are also presented in Figure 2 for PCBs categorized into readily cleared and slowly cleared congener groups according the structure activity rules for PCB persistence in birds described previously. Plasma clearance constants from both the readily cleared and the slowly cleared congener groups exhibited pronounced declining trends with increasing chemical hydrophobicity. The linear regression fit, slopes, and intercepts from regression analyses performed on log10-normalized $k_{pc}$ data versus log10 $K_{ow}$ for the two congener classes are presented in Figure 2. For both congener categories, the slopes were found to be significantly different (ANOVA; $p < 0.001$) than zero. Analysis of covariance indicated no significant differences ($p > 0.5$) in the slopes associated with the two linear regressions.

Plasma/fat distribution ratios ($K_{pf}$) were determined for each chemical using PCB concentrations determined in carcass homogenates (normalized to lipid content) divided by the corresponding concentration in plasma (wt/wt) of individuals sacrificed at 0 (summer), 148 (winter), and 348 (summer) d of elimination (Table 1). The $K_{pf}$ values for readily cleared congeners could be calculated only for individuals sacrificed at day 0 because of low or nondetected PCB levels found in plasma during the later time points. For the slowly cleared congeners, no significant differences (ANOVA; $p > 0.1$) were found among $K_{pf}$ values for winter birds compared to summer data...
birds. For all chemicals, the linear regression between log_{10} tissue concentrations in plasma versus log_{10} carcass fat PCB concentrations yielded a slope not significantly different than 1 (ANOVA; \( p > 0.10 \)). Figure 3 demonstrates the linearity of plasma versus fat PCB concentrations for six representative persistent congeners, which span a concentration range of four orders of magnitude. Inclusion of all chemicals produced an overall \( K_{\text{OW}} \) of 0.0060 ± 0.0001, although mean \( K_{\text{PF}} \)s for individual congeners varied by a factor of two, ranging from 0.0045 to 0.0094 (Table 1). Trends associated with \( K_{\text{PF}} \) for readily cleared and persistent congeners as a function of chemical \( K_{\text{OW}} \) are presented in Figure 2. Unlike \( k_{\text{spc}} \)s, no apparent association was observed between \( K_{\text{PF}} \) and chemical hydrophobicity. Linear regression analyses performed on \( K_{\text{PF}} \) versus log chemical \( K_{\text{OW}} \) yielded slopes that were not significantly different from zero for readily cleared (ANOVA; \( p < 0.2 \)) and persistent (ANOVA; \( p < 0.4 \)) congener groups. The \( K_{\text{PF}} \)s for the readily cleared PCBs, however, were found to be approximately 25% higher than \( K_{\text{PF}} \)s determined for slowly cleared congeners.

**Bioaccumulation of PCBs in American kestrels**

To further evaluate trends determined for PCB clearance using plasma concentration trends, the bioaccumulation potentials for individual PCBs determined at the end of the 120 d uptake period was assessed using relative biomagnification factors [16,21]. Relative biomagnification factors (BMF_{rel}(180)) were calculated according to

\[
\text{BMF}_{\text{rel}(180)} = \frac{C_{\text{f}(180)}}{C_{\text{oil}(x)}} \frac{C_{\text{oil}(180)}}{C_{(180)}}
\]

where \( C_{\text{f}(180)} \) and \( C_{\text{oil}(180)} \) are the concentrations of PCB congener \( x \) in the day 0 elimination carcass homogenates and dosing oil (ng/g), respectively. Both \( C_{(180)} \) and \( C_{\text{oil}(180)} \) are the concentrations of PCB180 in the carcass and oil samples (ng/g). The reference compound (PCB180) was chosen because of its high contribution in the dosing oil and because this congener had one of the slowest rates of elimination for the various congeners of study (Table 1). Figure 4 summarizes trends in BMF_{rel}(180) values for readily cleared and slowly cleared congeners as a function of chemical \( K_{\text{OW}} \). As observed for \( k_{\text{spc}} \)s, BMF_{rel}(180) values showed a separation of the readily cleared and slowly cleared PCBs. Relative biomagnification factors for both readily cleared versus slowly cleared congener groups were significantly (ANOVA; \( p < 0.001 \)) related to chemical \( K_{\text{OW}} \).

**DISCUSSION**

Trends observed for plasma clearance, whole body clearance, and relative biomagnification factors of individual PCBs suggest that these chemicals can be broadly classified into readily cleared versus slowly cleared congener groups on the basis of chlorine substitution patterns. Separation of PCBs into two persistence categories on the basis of presence of open *meta–para* sites on at least one of the phenyl rings have been reported in other studies using birds from field studies and from laboratory experiments [10,12,13,16,21]. The results of the present study validate the general structure activity relationships that describe PCB persistence in birds.
Characteristics of PCB plasma/fat partitioning in American kestrels

This study also provides calibrated toxicokinetic parameters necessary to describe PCB elimination in American kestrels using a two-compartment rate constant model. Plasma/fat partitioning coefficients are an important parameter that relate the concentrations of chemical in the central compartment to the major storage depot of the animal. The magnitude and range of $K_{pc}$ for PCBs determined in American kestrels (mean value of 0.0060 ± 0.0001) was similar to the magnitude and variability reported for $K_{pc}$ determined for other PHAHs in birds. For example, the mean $K_{pc}$ for p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE) obtained from field-collected American kestrels was 0.0044 (calculated from data presented in [30]). Similarly, in white-faced ibis, field-based $K_{pc}$ for p,p′-DDE were reported in the range of 0.0054 to 0.0084 [31], and in herring gulls, mean $K_{pc}$ for nine organochlorine chemicals ranged from 0.0039 to 0.0067 [5].

The high degree of consistency between plasma/fat partitioning among individual PCB congeners that exhibit a wide range of chemical hydrophobicities supports the general conclusions of Clark et al. [5] that plasma/fat distribution reflects equilibrium partitioning of contaminants between lipid pools of the two interacting tissues. Other studies have reported a decreasing trend in blood/fat partitioning with increasing degree of chlorination of PCBs for glaucous gulls [32] and marine mammals [33]. Differences in blood/fat partitioning trends of PCBs as reported in these studies may be associated with the use of different sample matrices (cellular fraction of blood vs plasma) and differences in the analytical methodologies employed. A second observation made in the present study was that the $K_{pc}$ values for readily cleared congeners were slightly but consistently elevated compared to $K_{pp}$ values for persistent congeners. Parham et al. [34] reported a similar trend for plasma/fat partitioning of readily cleared versus slowly cleared PCBs in humans. The elevated $K_{pp}$ values associated with readily cleared congeners suggests that the higher whole body elimination rates observed for these compounds can be partially explained by a lower storage capacity of adipose tissues for these congeners. However, the relatively small difference in $K_{pp}$ between the two persistence classes (25%) is not sufficient to explain the much greater differences observed between plasma clearance rates for the two congener groups. It is not clear why $K_{pc}$ for rapidly cleared PCBs were elevated compared to persistent congeners. It is possible that selective binding of readily cleared congeners to plasma components occurs. Further study on the distribution of readily cleared versus slowly cleared PCBs to various blood components should be investigated in order to test such a hypothesis.

Characteristics of PCB plasma clearance constants in American kestrels

The area under the plasma PCB concentration–time curves (AUCs) used to calculate $k_{pc}$ values were calculated for each congener using the trapezoidal rule according to Equation 7 and assuming a destructive sampling design (the mean $C_{p}$ from all birds sampled at a given time point was utilized in Eqn. 7) [29]. In addition, individual AUCs were determined in four birds that, by chance, were sampled repeatedly (at least three to five times), permitting calculation of individual AUCs using a composite design [29]. No differences were observed between the mean AUC derived from individual birds and the AUC derived from the destructive design for persistent congeners. The AUC derived from the destructive design was subsequently used to calculate congener-specific $k_{pc}$ values because this parameter integrated the largest number of individuals and time points.

No other studies that describe plasma clearance constants of individual PCBs by birds were found in the literature to compare with the results obtained here. However, the range of $k_{pc}$ calculated for PCBs in American kestrels were found to be similar to $k_{pc}$ determined for other PHAH compounds in herring gulls [5]. In the herring gull, readily cleared PHAHs such as hexachlorobenzene, octachlorostyrene, and dieldrin exhibited $k_{pc}$ in the range of 0.105 to 0.231 ml/g/d, whereas persistent PHAHs, which are resistant to metabolic attack (mirex, photomirex, p,p′-DDE, and oxychlordane), had $k_{pc}$ values on the order of 0.035 to 0.057 ml/g/d [5].

A surprising observation in the present study was the parallel trends observed between log $k_{pc}$ versus log $K_{ow}$ for PCBs in each persistence category. The most important mechanism contributing to high elimination rates observed for readily cleared PCBs is considered to be metabolic biotransformation [35,36]. Borlakoglu and Wilkins [37] demonstrated metabolic alteration of several readily cleared PCB congeners by microsomes isolated from PCB-exposed pigeons. It was therefore assumed that the high $k_{pc}$ values determined for readily cleared PCBs in the present study resulted from increased metabolic biotransformation of these congeners in the liver of the animals. For highly recalcitrant PCBs such as PCB153, which are subject to very slow metabolic attack, the major mechanism of whole body elimination in rats has been demonstrated to be fecal egestion of parent compound [38,39]. For American kestrels, it was assumed that elimination rates of slowly cleared compounds were governed by diffusive losses of parent PCBs from the animal to its feces. Under these assumptions, the experimental $k_{pc}$ for readily cleared congeners can be partitioned into two competing elimination processes:
where $k_{\text{met}}$ refers to the plasma clearance constant (ml/g/d) associated with metabolic biotransformation and $k_{\text{eg}}$ (ml/g/d) refers to the fecal egestion plasma clearance constant, which describes elimination of parent PCBs by diffusive losses. Given the lack of significant differences in slopes determined from linear regression analyses on log $k_{\text{pc}}$ versus log $K_{\text{OW}}$ for the two persistence groups, a single weighted average slope ($-0.387$) was calculated from the combined data, and new equations, forced to the weighted average slope, were derived to describe trends in $k_{\text{pc}}$ with chemical $K_{\text{OW}}$ using nonlinear regression techniques. The new equations are summarized as follows:

readily cleared PCBs:

$$\log k'_{\text{pc}} = \log(k_{\text{met}} + k'_{\text{eg}}) = -0.387 \cdot \log K_{\text{OW}} + 1.974;$$

$$R^2 = 0.79;$$

slowly cleared PCBs:

$$\log k'_{\text{pc}} = \log(k'_{\text{eg}}) = -0.387 \cdot \log K_{\text{OW}} + 1.425;$$

$$R^2 = 0.85;$$

Solving for $k_{\text{met}}$ and $k'_{\text{eg}}$ yields

$$k'_{\text{met}} = 67.582 \cdot K_{\text{OW}}^{-0.387};$$

$$k'_{\text{eg}} = 26.607 \cdot K_{\text{OW}}^{0.267};$$

Further combining Equations 9, 12, and 13 gives a generalized expression that allows prediction of plasma PCB clearance constants for all congeners:

$$k_{\text{pc}}' = 67.582 \cdot K_{\text{OW}}^{-0.387} (\text{MP}) + 26.607 \cdot K_{\text{OW}}^{0.267}$$

where MP = 1 for congeners having one or two open meta–para sites on the biphenyl ring and MP = 0 for the slowly cleared congeners having no open meta–para sites on the biphenyl ring.

Validation of this general equation was performed using Equation 14 to predict relative biomagnification factors and to compare these results with BMF$_{\text{rel}(180)}$ values determined in birds during the peak of the uptake period. The observed versus predicted regression equation explained over 87% of the variability of the data. For the readily cleared congeners, the observed BMF$_{\text{rel}(180)}$ values tended to cluster along the predicted line but exhibited much greater variability compared to persistent PCBs. Much of this variability was associated with $K_{\text{PF}}$ values measured for this class of congeners (Fig. 2). In Figure 4, the readily metabolized congener data were further normalized to account for variations in individual chemical $K_{\text{PF}}$ values by multiplying the observed BMF$_{\text{rel}(180)}$ value by the ratio of the average PCB $K_{\text{PF}}$ (0.0060) to the measured $K_{\text{PF}}$ for a given congener. The latter $K_{\text{PF}}$-normalized BMF$_{\text{rel}(180)}$ data are demonstrated to exhibit much less variability and conform more readily to the predicted relationships. This indicates that the deviation of $K_{\text{PF}}$ values measured for readily cleared PCBs have a measurable effect on the bioaccumulation potential of these compounds and do not represent a measurement artifact.

Factors regulating elimination and biomagnification of PCBs in American kestrels

The general agreement between predicted and measured BMF$_{\text{rel}(180)}$ values indicates that biomagnification factors of PCBs in American kestrels are controlled by elimination kinetics as opposed to differences in whole body uptake rate constants for the different congeners of study and that BMF$_{\text{rel}(180)}$ values are related to the congener chlorine substitution pattern and chemical hydrophobicity. Supporting evidence for the observed trends in BMF$_{\text{rel}(180)}$ values with chemical hydrophobicity can be derived from examination of biomagnification factors reported for slowly cleared PCBs in fish-eating sea birds. Braune and Norstrom [15] demonstrated two- to sixfold elevated BMFs of persistent octachlorobiphenyls relative to PCB74 in herring gulls. Similarly, Guruge and Tanabe [16] demonstrated fivefold elevated BMFs of hepta- and octachlorobiphenyls compared to persistent PCB60 in common cormorants. These results are similar to the three- to fivefold differences predicted for BMF$_{\text{rel}(180)}$ values in kestrels for the same congeners.

The data from the present study provide the first reported experimental evidence to indicate that biomagnification potentials and elimination rates of readily cleared PCBs in birds are $K_{\text{PF}}$ dependent. The factors controlling PCB elimination of readily cleared congeners are hypothesized to result from competing processes of metabolic biotransformation versus fecal egestion of parent PCBs. The fitted coefficients of Equation 14 indicate that both these processes are regulated by chemical hydrophobicity. For PCBs of similar hydrophobicity, readily cleared congeners will have elimination rates that are 3.54-fold higher than elimination rates of slowly cleared congeners. It is presently unclear why the two different elimination routes follow such consistent trends with chemical $K_{\text{PF}}$. Further experimentation to quantify the relative roles of biotransformation versus diffusion losses of PCBs to feces should be investigated in order to further consider the physiological interpretation of the empirical coefficients associated with Equation 14.

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