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Key Amino Acids in the Aryl Hydrocarbon Receptor Predict Dioxin Sensitivity in Avian Species

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Dioxin-like compounds are toxic to most vertebrates, but significant differences in sensitivity exist among species. A recent study suggests that the amino acid residues corresponding to Ile324 and Ser380 in the chicken aryl hydrocarbon receptor 1 (AHR1) are important determinants of differential biochemical responses to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in chickens and common terns. Here, we investigate whether the identity of these amino acid residues can predict embryonic sensitivity to dioxin-like compounds in a wide range of birds. AHR1 sequences were determined in species for which sensitivity data were available. Of all the species surveyed, chickens were unique in having the Ile/Ser genotype and were also the most sensitive to dioxin-like compounds. Turkeys, ring-necked pheasants, and Eastern bluebirds (intermediate Ile/Ala genotype) were less sensitive than chickens but more sensitive than American kestrels, common terns, double-crested cormorants, Japanese quail, herring gulls, or ducks (Val/Ala genotype). Our work suggests that key amino acids in the AHR1 ligand binding domain are predictive of broad categories of dioxin sensitivity in avian species. Given the large degree of variation in species sensitivity and the paucity of species-specific toxicity data, a genetic screen based on these findings could substantially improve risk assessment for dioxin-like compounds in wild birds.

Introduction

Dioxins, dibenzofurans, and polychlorinated biphenyls (PCBs) are lipophillic environmental contaminants that can adversely affect the health of wildlife and humans. Collectively referred to as dioxin-like compounds, these chemicals share structural similarities and a common mode of action. Dioxin-like compounds bind with high affinity to the aryl hydrocarbon receptor (AHR), a ligand-activated nuclear transcription factor. It is commonly held that major forms of dioxin toxicity occur through AHR activation (*1*).

Toxic effects of dioxin-like compounds have been described extensively in laboratory animals (*2*), with similar effects appearing in wildlife. Population declines in colonial fish-eating birds in the 1960s were largely attributed to exposure to high levels of PCBs (*3*). In laboratory tests, birds are particularly sensitive to dioxin-like compounds at the embryonic stage (*4*), and toxic potency varies substantially among chemicals. The most potent compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has a median lethal dose (LD50) of 0.3 μg/kg (ppb) in chickens exposed at embryonic day 7 for 72 h. Non-*ortho* substituted PCBs such as 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) and 3,3′4,4′-tetrachlorobiphenyl (PCB 77) have a similar degree of potency in the ppb range (3.1 and 8.6 ppb, respectively). The potency of mono-*ortho* substituted PCBs such as 2,3,3′,4,4-pentachlorobiphenyl (PCB 105), 2,3,3′,4,4′,5-hexachlorobiphenyl (PCB 156), and 2,3,3′,4,4′,5′-hexachlorobiphenyl (PCB 157) is more than 1000 times less with LD50s at the ppm level (*5–7*).

Embryotoxic effects of the most potent dioxin-like compounds are well-described in chicken, but less is known about the relative toxicity of these chemicals in wild birds. The data that do exist suggest that there are order of magnitude differences in the potency of individual compounds among species and that chickens are particularly sensitive (*4, 8*). For example, chickens are considerably more sensitive to PCBs than herring gulls, double-crested cormorants, or common terns—fish-eating birds with high environmental exposures to lipophillic contaminants. In laboratory tests, injection of 1000 μg/kg PCB 77 into fertilized herring gull eggs early in development had no effect on mortality, whereas in chicken embryos, a 50-fold lower dose was 100% lethal, and a 200-fold lower dose was 55% lethal (*9*). This differential sensitivity is also apparent in nature where Great Lakes herring gull populations maintain high reproductive success despite average egg concentrations of 3.2 μg/kg TCDD equivalents (lipid weight, converted from 2001 data for 14 Great Lakes herring gull colonies (*10*) using avian toxic equivalency factors (TEFs) (*11*)). Similar concentrations would result in higher than 50% mortality to chicken embryos.

The AHR has emerged as a key regulatory protein contributing to differential sensitivity to dioxin-like compounds in several animal models (*12*). In birds, there are at least two forms of AHR. Avian AHR1 is orthologous to the mammalian AHR and has a high affinity for dioxin-like compounds. More recently a second AHR isoform (AHR2) was identified in great cormorant and black-footed albatross (a predicted AHR2 is also found in the chicken genome) (*13, 14*). The role of avian AHR2 in dioxin toxicity has not been established, but preliminary evidence suggests that mRNA expression, affinity for TCDD, and transcriptional efficacy occur at levels below that of AHR1 (*15*).

The molecular basis for differential sensitivity to dioxin-like compounds in birds is not well understood, but a recent study suggests that, as in mammalian models, the AHR plays an important role. Karchner et al. (*16*) identified two amino acids in the avian AHR1 ligand binding domain (LBD) that may contribute to differential responses to dioxin-like compounds in chicken and common tern. Amino acid residues Ile324 and Ser380 in the high-affinity chicken AHR1 are replaced by Ala and Val in the low-affinity common tern AHR1. Using site-directed mutagenesis the authors were able to show that replacing Val or Ala in the common tern AHR1 with the corresponding chicken residues conferred a chicken-like high-affinity phenotype to the tern receptor. Considering the central role of AHR in dioxin toxicity, these amino acid differences may also contribute to the 260-fold difference in embryo lethal effects of PCB 126 in these species (*17*).

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Here we expand upon the Karchner et al. result by exploring the possibility that key amino acids in the avian AHR1 are predictive of broad categories of dioxin sensitivity across species. Comparative estimates of dioxin sensitivity were established in 12 species of birds for which egg injection data were available. These sensitivity data were assessed in relation to the predicted amino acid sequence of the AHR1 LBD for each species.

Materials and Methods

Review of Egg Injection Data. Relative dioxin sensitivity values for avian species were established based on a review of egg injection studies. Each value was expressed relative to chicken, the most sensitive species tested to date. All studies included in the review met the following criteria: (1) injection occurred during the first third of the incubation period, (2) the test compound was one of three potent dioxin-like chemicals—TCDD, PCB 126, or PCB 77, and (3) embryos were monitored until at least one day prehatch. In order to minimize the effect of between-study differences in other aspects of experimental design (injection site, injection day, vehicle, incubation position), the sensitivity of each species was expressed relative to that of chicken using within-study comparisons whenever possible. In cases where chicken and the species of interest were not tested in the same study (or set of studies), a reference chicken LD50 value was used to make the comparison.

Chicken LD50 reference values were established for PCB 126 and TCDD based on a comprehensive review of chicken egg injection literature (a reference value for PCB 77 was not required). These reference values are presented as the mean of LD50 values reported in independent studies. Only LD50 values derived from studies that tested multiple doses of the test compound and reported consistent concentration-dependent increases in mortality reaching 100% were included. Because there are considerably more egg injection data available for chicken than for other avian species, these limitations were applied to the chicken reference values only and not to other data under review.

For species other than chicken, all available peer-reviewed data derived from studies that matched the three criteria listed above were considered in our analysis. Because wild bird embryos are difficult to work with, the data generated from many of these egg injection studies were not ideal for making interspecies comparisons. In some cases the mortality in the vehicle group was extremely high (>40%), and in other cases the range of doses tested was not sufficient to establish a reliable LD50 value. Direct comparisons between LD50 values were made whenever possible. In cases where no LD50 was available relative species sensitivity values were derived from lowest observable adverse effect levels (LOAEL), no observable adverse effect levels (NOAEL), and LD100 values. Because these parameters are strongly influenced by which concentrations the researcher chooses to test, direct comparisons were not possible. Instead we made two assumptions that allowed us to increase the number of species for which we could make relative sensitivity estimates. The assumptions were as follows: (1) the LD50 falls between the reported LOAEL and the reported LD100 and (2) the difference between the reported NOAEL for a given species and the reported LD50 or LOAEL for the reference species (chicken) underestimates the difference between the LD50s for these two species.

AHR1 LBD Sequencing. A region of cDNA overlapping the LBD of chicken AHR1 (amino acid residues 235–402) was cloned from 9 species of birds. Five additional sequences were publicly available on GenBank. Frozen liver tissue from 5 species was retrieved from the specimen bank at the National Wildlife Research Centre (NWRC, Environment Canada, Ottawa, ON). These species were as follows: American kestrel (*Falco sparverius*), common eider (*Somateria mollissima*), double-crested cormorant (*Phalacrocorax auritus*), wood duck (*Aix sponsa*), and Japanese quail (*Coturnix japonica*). The specimen bank samples were collected by various Canadian Wildlife Service scientists over several decades and archived as studies ended. Archived samples were stored at −40 °C, or in liquid nitrogen, but in many cases previous handling was unknown. Eastern bluebird (*Sialia sialis*) and mallard (*Anas platyrhynchos*) liver tissues were kindly donated by Tim Fredricks. Fertilized ring-necked pheasant (*Phasianus colchicus*) and wild turkey (*Meleagris gallopavo*) eggs were obtained from a local breeder and sacrificed at one day prehatch. Livers were removed and immediately placed in RNA-later stabilization buffer (Ambion, Austin, TX, U.S.A.). Liver tissue was obtained from three individuals of each species except for mallard and turkey for which one and two livers were obtained, respectively.

Approximately 30 mg of whole liver tissue from each individual was homogenized using QIAshredder mini columns (Qiagen, Mississauga, ON). Total RNA was isolated from homogenized samples with the RNeasy Mini kit (Qiagen). RNA was quantified by spectrophotometric analysis at 260 nm, and 3 μg of RNA was reverse transcribed to cDNA using Superscript II and random hexamer primers (Invitrogen Canada, Burlington, ON). The cDNA samples were PCR amplified with forward primer 5′-CCAGACCAACTTCCTC-CAGA-3′ and reverse primer 5′-CACCAGTGGCAAACAT-GAAG-3′, for 35 cycles using 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s. PCR products run on an agarose gel resulted in a single band of approximately 600 bp for all species. This band was excised, purified with the QIAquick gel extraction kit (Qiagen), and then cloned using the TOPO TA Cloning kit (Invitrogen). Plasmid DNA was purified with the QIAprep spin miniprep kit (Qiagen) and sequenced on an Applied Biosystems 3730 DNA Analyzer at the Ontario Health Research Institute (OHRI, Ottawa, ON). A minimum of three plasmids originating from three separate bacterial colonies were sequenced for each species. In most cases one plasmid was sequenced for each of the three individuals. Because there were fewer than three liver samples for wild turkey and mallard, three plasmids were sequenced for each individual in these species.

Sequence Analysis. Nucleotide sequences were translated using Expasy online translation tool. Alignments of nucleotide and amino acid sequences were made using ClustalW software. Amino acid alignments identified residues that differed between species and individuals. A minimum of zero and a maximum of 4 residues varied among individuals of the same species. The vast majority of these variable amino acids were at conserved positions and are most likely a result of PCR error. The amino acid residues corresponding to Ile324 and Ser380 in chicken were not variable among individuals for any of the species surveyed.

Nucleotide alignments were used to establish a consensus sequence between individuals of the same species and to determine if any two sequences from different species were identical (indicating possible problems with contamination). Reported data indicate consensus between at least two out of three sequences at the nucleotide level. Reported amino acid sequences were generated from the consensus nucleotide sequence.

Results

Review of Egg Injection Data. Chicken LD50 reference values of 0.18 μg/kg TCDD and 1.1 μg/kg PCB 126 were established based on a review of egg injection data. Studies included in the review varied with respect to injection day (day 0 or day 4), end point (hatch or embryonic day 19), vehicle (emulsion, triolein, corn oil, or acetone), and injection location (yolk or air cell). Despite these differences, LD50 values were fairly consistent across experiments. For TCDD, the LD50s used

TABLE 1. Relative Sensitivity of Avian Species to TCDD, PCB 126, and PCB 77

species name	relative sensitivity[a]	data used to determine relative sensitivity
chicken	1	By definition, the relative sensitivity value for chicken is 1. Reference chicken LD50 values are 0.18 μg/kg TCDD and 1.1 μg/kg PCB 126.
ring-necked pheasant (RNPH)	12[b]	LD50 is 2.2 μg/kg TCDD in RNPH (*38*). This value was compared to the chicken TCDD reference value.
Eastern bluebird (EABL)	6–56	LD50 is between 1 μg/kg TCDD (NOAEL) and 10 μg/kg TCDD (100% mortality) in EABL (*39*). This range was compared to the chicken TCDD reference value.
turkey (WITU)	18–55	LD50 is between 20 μg/kg PCB 126 (LOAEL) and 60 μg/kg PCB 126 (100% mortality) in turkey (*40*). This range was compared to the chicken PCB 126 reference value.
double-crested cormorant (DCCO)	27[b,c]	LD50 is 4 μg/kg TCDD in DCCO and 0.15 μg/kg TCDD in chicken (*19, 41*).
double-crested cormorant (DCCO)	77[b]	LD50 is 177 μg/kg PCB 126 in DCCO and 2.3 μg/kg PCB 126 in chicken (*19, 41*).
American kestrel (AMKE)	122[c]	LD50 is 316 μg/kg PCB 77 in AMKE, and 2.6 μg/kg PCB 77 in chicken (*17*).
American kestrel (AMKE)	163[b]	LD50 is 65 μg/kg PCB 126 in AMKE and 0.4 μg/kg PCB 126 in chicken (*17*).
common tern (COTE)	260	LD50 is 104 μg/kg PCB 126 in COTE and 0.4 μg/kg PCB 126 in chicken (*17*).
wood duck (WODU)	>46	No adverse effects were observed at 4.6 μg/kg TCDD in WODU. This value was compared to a chicken LD50 value of 0.1 μg/kg TCDD (*18*).
common eider (COEI)	>200	In a single-dose test, no adverse effects were observed at 1000 μg/kg PCB 77 in COEI. This value was compared to a chicken LOAEL of 5 μg/kg PCB 77 (*9*).
herring gull (HERG)	>200	In a single-dose test, no adverse effects were observed at 1000 μg/kg PCB 77 in HERG. This value was compared to a chicken LOAEL of 5 μg/kg PCB 77 (*9*).
Japanese quail (JAQU)	>218	In a single-dose test, no adverse effects were observed at 240 μg/kg PCB 126 in JAQU (*5*). This value was compared to the chicken PCB 126 reference value.
mallard (MALL)	>1000	No adverse effects were observed at 5000 μg/kg PCB 77 in mallard (also referred to as Swedish duck or Pekin duck, *Anas platyrhynchos*). This value was compared to a chicken LOAEL of 5 μg/kg PCB 77 (*9*).

[a] Species sensitivity to one of the three test compounds (TCDD, PCB 126, or PCB 77) relative to chicken. Higher numbers reflect lower relative sensitivities. [b] LD50 values derived from egg injection studies where mortality in the vehicle control group was particularly high (≥40%). [c] LD50 values derived from egg injection studies where the highest dose tested caused less than 90% mortality.

to calculate the reference value were 0.1 (*18*), 0.15 (*19*), 0.122, 0.297 (*20*), and 0.24 (*21*) μg/kg TCDD. Two other LD50 values (0.15 and 0.678 μg/kg TCDD) were omitted from the reference value calculation because mortality did not follow a concentration–response relationship near the middle of the curve (*22*). For PCB 126, the data used to calculate the LD50 reference value were 0.4 (*17*), 0.6 (*23*), and 2.3 (*19*) μg/kg PCB 126. Several other egg injection studies gave similar LD50 values for PCB 126 in chicken embryo: 1.5 (*24*), 1.01 (*25*), and 0.6 (*26*) μg/kg PCB 126. These were not used to calculate the reference value, because they did not include full dose–response curves.

Relative dioxin sensitivity values were estimated for 12 species (Table 1). Four types of comparisons were made: (1) direct comparisons between a chicken LD50 value and a test species LD50 value within a study (American kestrel, common tern and double-crested cormorant), (2) comparison between a chicken LD50 reference value and a test species LD50 value (ring-necked pheasant), (3) comparison between a chicken LD50 reference value and a range of values within which the test species LD50 would be expected to fall (Eastern bluebird and turkey), and (4) comparison between a chicken LOAEL or LD50 value and a NOAEL for a test species (common eider, domestic duck, herring gull, Japanese quail, and wood duck). Rankings of species sensitivity to dioxin-like compounds determined by this method corresponded well with rankings determined by other methods, including comparisons of NOAEL values (*18*), values determined based on TCDD equivalents (TEQs) (*27*), and data for induction of ethoxyresorufin-*O*-deethylase (EROD) activity in cultured hepatocytes (*28*).

AHR1 LBD Sequences. A region of cDNA overlapping the chicken AHR1 LBD (amino acid residues 235–402) was cloned and sequenced from 9 avian species. These sequences are available in GenBank under accession numbers EU660867 to EU660875. Alignment with publicly available sequences for chicken (GenBank Accession no. AF192502), common tern (AF192503), herring gull (DQ371287), great cormorant (AB109545), and black-footed albatross (AB106109) demonstrated that this region of the AHR1 is highly conserved among avian species. The percent identity at the amino acid level was above 97% for all species comparisons, and only 6 amino acid residues in the AHR1 LBD were variable among the 14 available sequences (Figure 1). The percent identity for alignment of cloned fragments with the corresponding region of great cormorant AHR2 (AB287294) or black-footed albatross AHR2 (AB106110) was much lower, indicating that we had cloned AHR1 and not AHR2.

Correspondence between AHR1 LBD Sequence and Relative Sensitivity. Three combinations of the amino acids corresponding to Ile324 and Ser380 in the chicken AHR1 were observed among the species surveyed: Val/Ala, Ile/Ala, and Ile/Ser. A consistent relationship between the identity of these amino acids and relative sensitivity to TCDD, PCB

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chicken   235 AMNFQGRLKFLHGQNKKGKDGAALSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF 294
EABL          AMNFQGRLKFLHGQNKKGKDGATLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
BFAL          AMNFQGRLKFLHGQNKKGKDGATLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
RPHE          AMNFQGRLKFLHGQNKKGKDGTALSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
WITU          AMNFQGRLKFLHGQNKKGKDGAALSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
COEI          AMNFQGRLKFLHGQNKKGKDGTTLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
MALL          AMNFQGRLKFLHGQNKKGKDGTTLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
WODU          AMNFQGRLKFLHGQNKKGKDGTTLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
HERG          AMNFQGRLKFLHGQNKKGKDGATLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
GRCO          AMNFQGRLKFLHGQNKKGKDGATLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
COTE          AMNFQGRLKFLHGQNKKGKDGATLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
DCCO          AMNFQGRLKFLHGQNKKGKDGATLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
AMKE          AMNFQGRLKFLHGQNKKGKDGATLSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
JAQU          AMNFQGRLKFLHGQNKKGKDGAALSPQLALFAVATPLQPPSILEIRTKNFIFRTKHKLDF
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                                        324
chicken   295 TPTGCDAKGKIVLGYTEAELCMRGTGYQFIHAADMLYCAENHVRMMKTGESGMTVFRLLT 354
EABL          TPTGCDAKGKIVLGYTEAELCMRGTGYQFIHAADMLYCAENHIRMMKTGESGMTVFRLLT
BFAL          TPTGCDAKGKIVLGYTEAELCMRGTGYQFIHAADMLYCAENHVRMMKTGESGMTVFRLLT
RPHE          TPIGCDAKGKIVLGYTEAELCMRGTGYQFIHAADMLYCAENHVRMMKTGESGMTVFRLLT
WITU          TPIGCDAKGKIVLGYTEAELCMRGTGYQFIHAADMLYCAENHVRMMKTGESGMTVFRLLT
COEI          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
MALL          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
WODU          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
HERG          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
GRCO          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
COTE          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
DCCO          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
AMKE          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
JAQU          TPTGCDAKGKIVLGYTEAELCMRGTGYQFVHAADMLYCAENHVRMMKTGESGMTVFRLLT
              **+*************************+************+******************

                                        380
chicken   355 KENRWAWVQANARLVYKNGRPDYIISTQRPLTDEEGAEHLRKRNMKL 401
EABL          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
BFAL          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
RPHE          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
WITU          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
COEI          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
MALL          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
WODU          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
HERG          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
GRCO          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
COTE          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
DCCO          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
AMKE          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
JAQU          KENRWAWVQANARLVYKNGRPDYIIATQRPLTDEEGAEHLRKRNMKL
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FIGURE 1. Amino acid alignment of the avian AHR1 LBD. Asterisks (*) indicate a match between all 14 sequences. Plus signs (+) indicate a mismatch. Residues corresponding to the amino acids that have been associated with functional differences between chicken and common tern AHR1s (*16*) are highlighted. Species codes are as described in Table 1 with the exception of two species for which no sensitivity data were available. These are black-footed albatross (BFAL) and great cormorant (GRCO).

126, or PCB 77 was observed among the 12 species for which toxicity data were available (Figure 2). Chickens were most sensitive to each test compound and had an Ile/Ser genotype. Moderately sensitive species had an Ile/Ala genotype, and insensitive species had a Val/Ala genotype. We did not observe a correspondence between the identity of the remaining four variable amino acids (Ala256, Ala257, Thr297, and Val337 in chicken) and dioxin sensitivity among the species surveyed.

Discussion

Sensitivity to dioxin-like compounds can vary by more than 1000-fold among different species of birds (Table 1). This variability is problematic for ecological risk assessment, because toxicity data are available for only a very limited number of species. Moreover, for all but the most commonly studied compounds, toxicity has been assessed exclusively in chicken—a particularly sensitive species that is most often an inappropriate model for wild birds.

One approach to improving risk assessment for dioxin-like compounds in avian wildlife would be to generate more toxicity data for ecologically relevant species. To this end, several egg injection experiments are presently being carried out by our group and others. However, problems inherent to toxicity testing in wild birds—namely low hatching success for artificially incubated eggs and limited availability of samples—contribute to making these data less than ideal, and other approaches are also needed. Here, we take a molecular approach and describe a correspondence between the identity of key amino acids in the AHR1 LBD and dioxin sensitivity in birds.

Review of Avian Toxicity Data. Previous reviews of the effects of dioxin-like compounds on avian species have focused on establishing threshold exposure levels that would

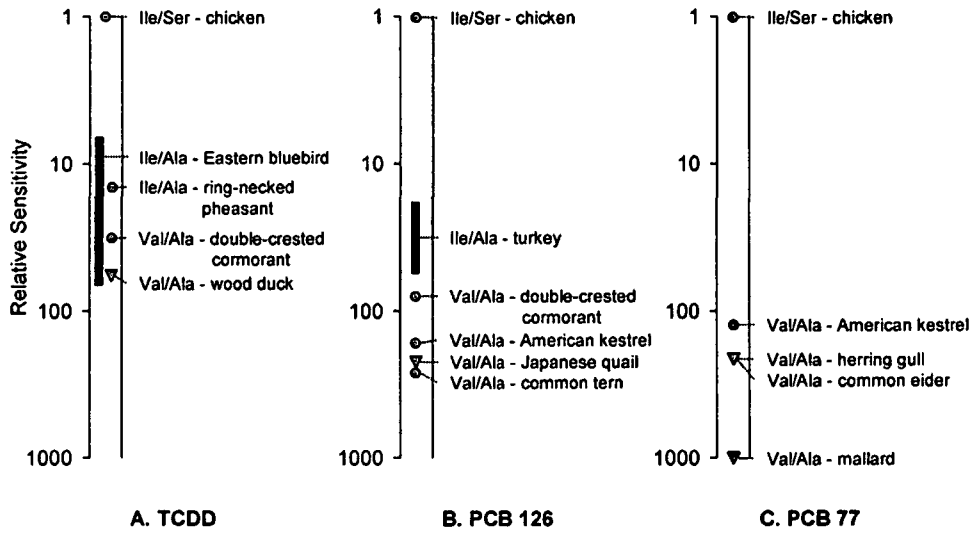

FIGURE 2. Correspondence between relative sensitivity to dioxin-like compounds (Table 1) and AHR1 genotype (Figure 1). Relative sensitivity values were derived from egg injection studies that tested: A) TCDD, B) PCB 126, or C) PCB 77. Values for each species are expressed relative to the most sensitive species, chicken, which is assigned a value of 1. Higher numbers represent lower sensitivities. Because of the heterogeneous nature of the data, relative sensitivity values are expressed in one of three ways: 1) circles represent a discrete estimate of relative sensitivity (determined from an LD50 value), 2) bars represent a range of values that the relative sensitivity would be expected to fall within (determined from a NOAEL or LOAEL and an LD100 value), and 3) triangles represent a value that is expected to underestimate relative sensitivity (determined from a NOAEL). See Table 1 for further details and references. Each data point is labeled with the species name and corresponding AHR1 genotype for that species. AHR1 genotype refers to the identity of the amino acids corresponding to Ile324 and Ser380 in the chicken AHR1.

be likely to cause adverse effects in wildlife (*4, 8, 29*). Here, we reviewed the literature with the particular purpose of establishing comparative estimates of species sensitivity for birds. For a number of reasons, egg injection studies were chosen as most appropriate for this purpose. Cross-species comparisons are facilitated by experimental constraints inherent in working with eggs. For example, the timing and duration of the exposure are tied to the developmental stage of the embryo and can be easily compared between studies (since most exposures start at embryonic day 0 and are carried through until hatching). Additionally, these data are relevant to ecological risk assessment because egg injection mimics maternal deposition of contaminants, and embryonic development is a sensitive life stage.

We reviewed studies that tested one of three compounds: the prototypical dioxin-like compound, TCDD, and two non-*ortho* substituted PCBs, PCB 126 and PCB 77. Comparisons between test compounds were avoided because it is not clear if the relative toxicity of TCDD, PCB 126, and PCB 77 is consistent across species. For example, PCB 77 is 50−100 times less potent than TCDD as an inducer of EROD activity (an AHR-mediated response) in chicken, ring-necked pheasant, and turkey embryo hepatocyte cultures but more than 1000-times less potent than TCDD in Pekin duck and herring gull embryo hepatocytes (*28*). These data may partially explain the absence of a toxic response to high concentrations of PCB 77 in herring gulls and ducks (Table 1) and highlight the complexity of carrying out ecological risk assessment for mixtures of chemicals in wild species. The toxicity of individual compounds and the sensitivity of individual species can both vary by up to 1000-fold. In addition, the potency of each compound relative to TCDD is not necessarily consistent among species. A clearer understanding of the molecular mechanisms underlying differences in the potency of individual compounds in individual species is needed.

Basis for Predicting Dioxin Sensitivity from AHR Genotype. Given the central role of the AHR in dioxin toxicity, associations between the affinity with which a compound binds to the receptor, the subsequent biochemical response, and *in vivo* toxicity have been proposed by several researchers. Brunström hypothesized that the differences in species sensitivity described in his egg injection experiments might be related to differential AHR binding affinity or expression levels (*30*). This hypothesis is consistent with later findings; relative to chicken, AHR binding affinity is lower by approximately 15-fold in double-crested cormorant (*31*) and 7-fold in common tern (*16*). Both species are also less sensitive to the embryotoxic effects of dioxin-like compounds (Table 1). It has also been suggested that downstream biochemical effects of AHR activation might be useful for predicting *in vivo* responses to dioxin-like compounds. In chickens, the potency of PCB 169, PCB 77, and PCB 126 to induce EROD activity in avian liver corresponded to the rank order embryotoxicity of these compounds (*6*). Kennedy et al. (*28*) suggest that species that are more sensitive to EROD induction in embryo hepatocyte cultures may also be more sensitive to the lethal effects of dioxin-like compounds assessed via egg injection experiments.

More recently, the work of Karchner et al. (*16*) has uncovered a mechanistic link between the amino acid sequence of the AHR1 and the degree to which the receptor is activated by TCDD. Residues Ile324 and Ser380 in the high affinity chicken receptor are replaced by Val and Ala in the low affinity common tern receptor. Using site-directed mutagenesis, the authors demonstrated that these substitutions are responsible for a 7-fold difference in TCDD binding affinity and reduced transactivation by the tern AHR1 expressed in COS-7 cells. This finding may partially explain the 80-fold difference in the inducibility of EROD activity in cultured hepatocytes (*32*) and the 260-fold difference in embryolethality of PCB 126 (*17*) in chickens and common terns. Interestingly, the amino acid residues corresponding to Ile324 and Ser380 in the chicken are also important determinants of dioxin sensitivity in a mouse model (*16, 33−35*).

In the current study, we investigated whether key amino acid residues corresponding to Ile324 and Ser380 in the chicken AHR1 are sufficient for predicting categories of dioxin sensitivity in diverse avian species. Of the species we surveyed, chickens were most sensitive to all three test compounds

and were the only species with an Ile/Ser genotype (Figure 2). Species that were least sensitive to each of the test compounds had a Val/Ala genotype similar to common tern. A third group of species was moderately sensitive to PCB 126 or TCDD and had an intermediate Ile/Ala genotype. Additional egg injection data generated by Brunström and colleagues suggest that turkeys, an Ile/Ala species, are also moderately sensitive to PCB 77. These data were not included in Table 1 because they did not permit any of the four types of comparisons described in the results section. Nevertheless, in this study, a dose of 1000 μg/kg PCB 77 caused 60% mortality to turkey embryos, while a 20-fold lower dose caused 100% mortality to chicken embryos (*36*). In contrast, 1000 μg/kg PCB 77 caused no observable effects in common eiders, herring gulls, or mallards (Table 1). *In vitro* data also suggest that turkeys might be moderately sensitive to PCB 77. Turkeys were over 300 times more responsive than herring gulls and 20 times less responsive than chickens to EROD induction by PCB 77 in cultured hepatocytes (*28*).

Interestingly, the sensitivity classifications and AHR1 genotypes shown in Figure 2 do not always correspond to phylogenetic relationships among the species surveyed. For example, four species of Galliforms (chicken, ring-necked pheasant, turkey, and Japanese quail) have three different AHR1 LBD genotypes corresponding to three levels of sensitivity. The evolutionary significance of variation in AHR genotype and species sensitivity to dioxin-like compounds warrants further investigation.

Our results suggest that, as in other animal models, the amino acid residues corresponding to Ile324 and Ser380 in the chicken AHR1 are important determinants of dioxin sensitivity in birds. Four additional amino acid residues in the AHR1 LBD were also variable among the species surveyed. We did not observe any clear correspondence between these and dioxin sensitivity in the current study, but the contribution of each variable amino acid to ligand binding affinity and a subsequent toxic response warrants further investigation. Areas of the AHR1 outside of the LBD or other molecules involved in the dioxin response pathway are also likely to contribute to species differences in sensitivity. This is suggested by the observation that American kestrels are more sensitive to PCB 77 than herring gulls (Table 1) even though the AHR1 LDB is 100% identical at the amino acid level in these two species. Additionally, the toxic response to dioxin-like compounds is not just a molecular one; species differences in uptake, tissue distribution of contaminants, and metabolism may also be important factors determining sensitivity. For example, PCB 77 is known to be readily metabolized in birds (*37*). Species differences in biotransformation rates may account for some of the variation in the potency of this compound. Although the structure of the avian AHR1 LBD cannot fully explain all of the variance in dioxin sensitivity among different species of birds, the association between key amino acid residues in the AHR1 LBD, AHR affinity for dioxins, and embryotoxicity appears to be an important one with some predictive power. This finding suggests that AHR genotyping might be useful as a genetic screen for predicting species sensitivity to dioxin-like compounds in contaminated ecosystems.

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