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Review

Acute toxicity and metabolism of pesticides in birds

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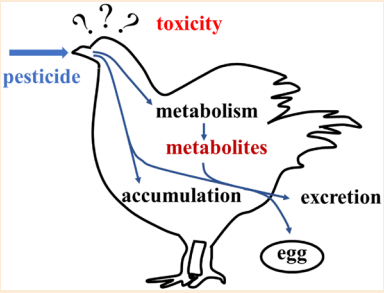
Supplementary material

The median lethal dose of pesticide in acute oral toxicity, used as a conservative index in avian risk assessment, varies by the species with differences of less than one order of magnitude, depending on body size, feeding habit, and metabolic enzyme activity. The profiles of pesticide metabolism in birds with characteristic conjugations are basically common to those in mammals, but less information is available on their relevant enzymes. The higher toxicity of some pesticides in birds than in mammals is due to the lower activity of avian metabolic enzymes. The bioaccumulation in birds is limited for very hydrophobic pesticides resistant to metabolic degradation. Several *in silico* approaches using the descriptors of a pesticide molecule have recently been employed to estimate the profiles of acute oral toxicity and bioaccumulation.



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Introduction

Many kinds of wild birds inhabit the agricultural and forest regions where pesticides are applied frequently. Birds may either inhale the air contaminated with pesticides or be exposed dermally *via* skin or during and after the spray application of pesticides. Contact with pesticide deposits on crops and weeds *via* the feathers may result in the oral uptake of pesticides by preening. Since crops, weeds, and insects are the general food for birds, the oral uptake of pesticide residues therein is most probable.[1)] When pesticides are applied as a seed dressing, birds may be exposed to pesticides at a higher concentration by ingesting the treated seeds. In the case of a granule formulation, birds mistake the granules for seeds or ingest them as grit.[1,2)] By taking account of these possible routes of exposure and the feeding habit of birds, the toxicological impact of pesticides should be assessed based on their intrinsic toxicity *via* direct administration. For this purpose, the competent authority of registration first requires data on the acute oral (a median lethal dose, LD_{50} in mg/kg)[3)] and additional dietary (a median lethal concentration, LC_{50} in ppm)[4)] toxicity of the pesticide in the standard species, such as the northern bobwhite quail (*Colinus virginianus*) as an upland game bird and the mallard duck (*Anas platrhynchos*) as a waterfowl. Generally, it is difficult to extrapolate the toxicity observed in these species to that in another wild species because of differences in body size, feeding habit, and physiology. These differences are supposed to affect the metabolic activity, which finally determines the local concentration of a pesticide at a toxicological target site. Incidentally, when pesticide residues are detected in the feed items of poultry, the data on the transfer of residues to muscle, fat, liver, and eggs should be examined to set appropriate tolerances. The registrant conducts the metabolism and residue studies of the pesticide in laying hens (*Gallus gallus domesticus*)[5)] to determine the terminal residues of the pesticide and its major metabolites with the metabolic pathway.

The first part of this review deals with the acute and short-term toxicity as the first tier of avian risk assessment, from the viewpoints of route of exposure, species differences, and bioactivation; it also introduces the progress of their *in silico* estimation. The possible bioaccumulation of the pesticide *via* the food web is briefly discussed in relation to its partition coefficient and metabolism. The relevant factors controlling the metabo-

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lism, such as the administration route, species differences, and chirality, are summarized in the second part, together with the metabolic profiles of the pesticides by chemical class. Finally, an overview summary is provided, including issues that should be examined for a more refined avian risk assessment.

1. Acute and short-term toxicity

1.1. Routes of exposure

In order to examine the contribution of each exposure route in birds, the inhibition of brain cholinesterase (ChE) activity was conveniently used for northern bobwhite quail kept in an environmentally controlled closed-loop wind tunnel, where the aerial application of methyl parathion (MP) to cotton plants was simulated.[6] The inhibition in the untreated birds represents that *via* all exposure routes. Birds covered with a body bag with only the beak being exposed to air, wearing a neck collar, or fed MP-treated meal worms were used to examine the contribution of inhalation, oral route by preening, or dietary route, respectively. The extent of dermal exposure was estimated by subtracting the contribution of each route above from that of all routes to the inhibition in the untreated birds. Exposure *via* inhalation and preening was critical only in the early period after aerial application; thereafter, both oral and dermal exposure routes became dominant. The importance of dermal exposure was also reported for brown-headed cowbirds in the semi-field study with the spray application of azinphos-methyl to apple trees.[7] Incidentally, standardized guidelines of acute oral toxicity are available, and the pesticide is singly administered either by gelatin capsule or gavage at five to ten doses with a limit of 2000 mg/kg.[3] In contrast, an acute dermal toxicity method still is not standardized, and the toxicity seems to depend on the site of application. The dermal toxicity of organochlorine (OC), organophosphorus (OP), and carbamate pesticides was compared among several avian species, when the acetone solution of each pesticide was applied to their foot pads or sparsely feathered breast under their wings.[8] Lower LD_{50} values were obtained for the breast application, possibly due to more absorption of pesticide through the thinner stratum corneum of the breast than that of the foot pads. Incidentally, Hudson *et al.*[9] showed moderate correlation between oral and dermal toxicity ($r=0.65$) for 20 pesticides applied to the foot pads of mallard ducks. They also introduced the dermal toxicity index, $DTI=[LD_{50}\ (\text{oral})/LD_{50}\ (\text{dermal})]\times 100$. Most of the tested pesticides showed values of less than 100, indicating lower toxicity *via* the dermal route than *via* the oral one. This trend was later confirmed for 19 pesticides by using seven avian species, and the DTI value was found to be pesticide-specific and independent of avian species.[8]

These studies may show that acute oral toxicity is adequate as a primary index, but the other exposure routes should be kept in mind when assessing the toxic potential of pesticides in the field. Mineau[10] analyzed the impact scoring data in the field monitoring of ChE-inhibiting pesticides ($n=35$) by using a logistic regression model. The 5% hazardous dose of the oral toxicity in the avian species-sensitivity distribution (HD_5) was always an important factor for the "kill or no-kill" classification of pesticides. Both the DTI value and Henry's law constant of a pesticide were necessary for its more precise classification, showing the importance of the dermal and inhalation routes in the field.

1.2. Acute oral toxicity

The short-term dietary toxicity study is another registration requirement. According to the standardized guidelines,[4] birds are daily fed a diet homogeneously treated with the pesticide, usually at five concentrations with a limit of 5000 ppm, for five consecutive days. Since there is some difficulty in properly determining the exposure, and the LC_{50} values weakly correlate with each other among test species, Mineau *et al.*[11] proposed the usage of LD_{50} instead for assessing the risk of a pesticide. Furthermore, Hilton *et al.*[12] recently conducted a retrospective analysis of 119 pesticides registered between 1998 and 2017 in the USA from the viewpoint of the risk quotient (RQ, a point estimate of exposure divided by LD_{50} or LC_{50}). The acute oral RQ was greater than the short-term dietary RQ in all but one case, and it could be used as the primary metric in comparison to the US EPA level of concern for avian risk assessment. On the basis of these examinations, acute oral toxicity is considered more appropriate as a conservative index.

In conducting an acute toxicity study, the adequacy of a pen-reared strain as the representative of its wild counterpart should be confirmed at least for standard species in the guidelines. By using northern bobwhite quail, this adequacy was demonstrated by similar brain ChE sensitivities against methyl paraoxon, hepatic microsomal oxidase activity on MP, and the acute oral toxicity of MP.[13] Incidentally, the acute oral toxicity data of pesticides are not always available for non-standard species; hence, any kind of extrapolation is highly desired. Since a chemical's uptake, distribution, and metabolism in mammals are highly related to their body weight, an allometric factor (AF) is generally used to extrapolate LD_{50}. This approach, using the function of $(\text{body weight})^{AF}$, has been successfully adopted for determining avian acute oral toxicity.[14] Regression analysis of the LD_{50} values in $\geq$10 avian species calculated the mean AF value to be 1.148 for 36 pesticides.

1.3. Bioactivation

Toxic symptoms of OC pesticides arise from their direct interaction with a target site, for example, axonal voltage–dependent Na channels for DDT and a GABA receptor for cyclodienes.[15] OPs and carbamates inhibit acetyl ChE (AChE) at postsynaptic membranes in nervous systems by covalently binding with the AChE serine residue. However, the oxidative bioactivation of the thiophosphoryl moiety to the corresponding phosphoryl (oxon) is indispensable for the exhibition of toxicity of many OPs. Some examples of bioactivation, meaning higher toxicity of a metabolite than of the parent pesticide, are summarized in Table 1. The contribution of metabolism, including abiotic reactions such as hydrolysis and photolysis, to enhancing toxicity should be kept in mind when considering pesticide toxicity.

Table 1. Bioactivation of pesticides

Sp.[a)]	Pesticide[b)]	LD_{50}[c)]	Metabolite	LD_{50}	Transformation	Ratio[d)]
M	Acephate*	234	Methamidophos	29.5	hydrolysis	7.9
M	Carbosulfan	10	Carbofuran	0.76	hydrolysis	13
B	Chlorfenapyr*	34	AC303,268	25	*N*-dealkylation	1.4
M	Chlorothalonil	>2000	SDS-3701	158	hydrolytic dechlorination	>13
B	Fipronil	11.3	MB 46513	5.4	desulfinylation	2.1
B	Fluopicolide	>1744	M01	1171	*N*-debenzylation	>1.5
B	Malathion	359	Malaoxon	43	oxidative desulfuration	8.3
M	Trichlorfon*	36.8	Dichlorvos	7.78	dehydration	4.7

[a)] Avian species. M, mallard duck; B, northern bobwhite quail. [b)] Data from Ref. 21 and 22(*). [c)] Acute oral, in mg/kg. [d)] LD_{50} (pesticide)/LD_{50} (metabolite).

1.4. Species differences

1.4.1. Characteristics of avian anatomy and physiology

Differences in the toxicity of pesticides between birds and mammals are influenced by many things.[16)] Birds must take in food more rapidly than mammals of similar body size to maintain their higher body temperature (*ca*. 42°C; mammals, 37°C); as a result, birds may ingest more pesticide from contaminated food. Birds are oviparous animals, and their eggs additionally provide a characteristic excretory route for lipophilic pesticides, which exposes the next generation to the pesticide at an early developmental stage. Furthermore, the relative size of the liver to the body is smaller in birds than in mammals, and avian urine is voided into the cloaca with the possible reabsorption of metabolites from here in addition to the intestine. Finally, the existence of the coccygeal mesenteric vein connecting the hepatic portal vein to the renal one facilitates the transport of the pesticide and its metabolites through the blood stream from the gastrointestinal tract to both the liver and kidneys, which increases the contribution of metabolism in the avian kidney.

1.4.2. Interspecies differences

Higher toxicity in birds than mammals has been frequently reported for ChE-inhibiting pesticides, such as OPs and carbamates,[16–18)] while the opposite is known for pyrethroids.[19)] These species differences closely relate not only to the enzyme activity of esterases and oxidases[16,18,19)] but also to the sensitivity at the toxicological target site of each pesticide.[20)] In order to grasp species differences in acute oral toxicity, we surveyed the literature, European Food Safety Authority (EFSA) regulatory reports,[21)] and US EPA Reregistration Eligibility Documents.[22)] Toxicity data are mostly available for several standard avian species and rats. By using the rat and the lowest avian LD_{50} values in these information, pesticides (n=297; 85 insecticides, 91 fungicides, 121 herbicides) were classified as non-toxic (LD_{50}>2000 mg/kg) in both species (58%), non-toxic only in birds (11%) or rats (6%), and toxic in both species (LD_{50}<2000 mg/kg; 25%, n=74; Fig. 1). The linear regression analysis on pesticides toxic to both species showed a moderate correlation; log LD_{50} (birds)=0.85 log LD_{50} (rats)+0.087 (r=0.60). This is consistent with the relationship (r=0.71) reported between mallard ducks and rats for 20 pesticides.[9)] From the accumulated evidence on species differences, the higher avian toxicity of OPs and carbamates and lower toxicity of pyrethroids were also confirmed (Fig. 1). A slope of less than unity may show a higher toxic sensitivity in birds than in rats. Incidentally, no correlation was observed in acute dermal toxicity between mallard ducks (foot pad application) and rats, most probably due to the difference of structural proteins in the epidermal stratum (α-keratin in mammals and β-keratin in birds).[9)] When the breast application *via* its thinner stratum corneum was conducted for seven avian species, the correlation of DTI between birds and rats was found to be good (r=0.94), but it became much lower for pesticides requiring metabolic activation to exhibit toxicity.[8)]

1.4.3. Intra-species differences

The enzyme activities of ChE, carboxylesterase (CaE), and cytochrome P450 (CYP) depend on both the body size and feeding habit of birds.[16,23–25)] Uridine 5′-diphospho-glucuronosyltransferase (UDPGT) and sulfotransferase (SULT) are the metabolic enzymes participating in the conjugation of various metabolites, and their activity is also dependent on the avian species.[26,27)] Therefore, these differences may, at least in part, affect the acute oral toxicity in each species. The existing LD_{50} values in various

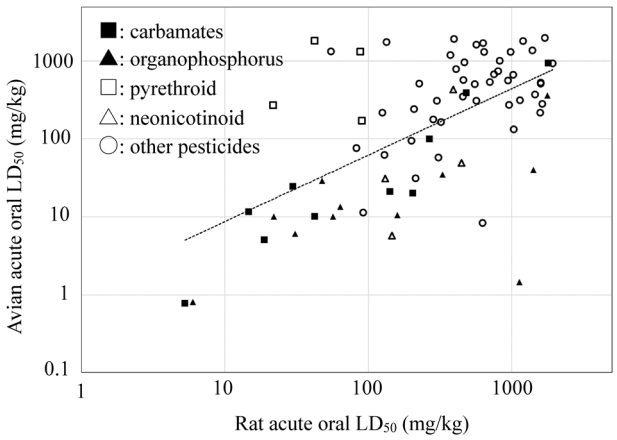

Fig. 1. Relationship of acute oral LD_{50} (mg/kg) between birds and rats. The lowest LD_{50} value of each pesticide on birds was taken from the reported data (see supplemental data).

species exhibit a wide range for each pesticide, pharmaceutical, and industrial chemical, but they mostly differ by less than one order of magnitude. For example, the median factors of differences in the reported LD_{50} values (number of avian species, chemicals) are calculated to be 2.3 (3, 17),[28] 3.1 (20, 24),[17] 3.3 (7, 25),[8] 3.9 (6, 17),[21] and 8.1 (6, 16).[29] Since the number of test species is very limited, usually 1 to 3, in the registration of a pesticide, together with the wide range of LD_{50} values described above, Mineau *et al.*[11] proposed using a species-sensitivity distribution method in the avian risk assessment of a pesticide, by considering the AF value of the body weight. They derived HD_5 as a toxicity reference value for each pesticide (n=880) on the basis of the acute oral LD_{50} data and tentatively identified 34 toxic pesticides as benchmarks, most of which are ChE inhibitors.

1.5. In silico *approach to acute toxicity*

The prediction of the acute oral LD_{50} value is very useful, not only for evaluating the potential avian risk of a pesticide candidate prior to its development but also for reasonably designing toxicity studies. *In silico* methods to describe the toxicity of pesticides have been applied less frequently to birds than to fish. Mazzatorta *et al.*[30] classified 113 chemicals by their acute oral toxicity in northern bobwhite quails, using QSAR analysis with a support vector machine and genetic algorithms. Both the molecular size/shape information and electrostatic properties on its surface were good descriptors relating to their transport process and interactions with a toxicological target site, respectively. Basant *et al.*[31] recently utilized decision tree forest or boost methods with nine descriptors of the molecular size, topology, and electronic properties, and they succeeded in describing the acute oral LD_{50} values of 131 pesticides in northern bobwhite quails (r^2=0.95–0.97). These tree-based QSAR models could also predict LD_{50} values in the mallard duck, Japanese quail, ring-necked pheasant, and house sparrow at a satisfactory level. Incidentally, several machine learning methods, including those mentioned above, have been applied to classify about 600 chemicals by their short-term dietary toxicity levels (LC_{50}) in the northern bobwhite quail and mallard duck.[32] The model using the support vector machine gave the best prediction of LC_{50} in both species, and the five most important molecular descriptors were related to the electro-topological state of a molecule relating to P and S atoms. Furthermore, the alert substructures for avian toxicity were suggested to be OP and organosulfur moieties, and the reactive anhydrides were suggested to be amidines and aryl bromides.

1.6. Bioaccumulation

Lipophilic pesticides with log K_{ow} $\geq$3 are susceptible to being accumulated from water, sediment, and soil to fish and sediment/soil-dwellers, both of which may be taken by birds as food.[1] Therefore, the bioaccumulation of pesticides by a dietary route, especially *via* the food web, should be assessed to protect avian species. For example, in a long-term feeding study of OCs using laying hens, high accumulation ratios (AR, the concentration ratio against food) of lindane, HCB, DDT, and dieldrin were observed in the fat (2–17).[33] In the case of poultry, the bioaccumulation potential of pesticides can be examined by the metabolism studies required for registration.[1] In a feeding study of hydrophobic chlordane in cockerels by a single dose, the physiologically based pharmacokinetic (PBPK) analysis clearly demonstrated that the effective metabolism after rapid absorption to the body results in insignificant accumulation in each organ, but with much slower elimination from fat.[34] Feeding studies of *beta*-cypermethrin in laying hens[35] and imidacloprid in Japanese quails[36] have also shown that the rapid metabolism of these pesticides results in the insignificant bioaccumulation of the parent insecticides and their metabolites in all tissues and eggs. In contrast, a similar analysis in the feeding study of chlordecone using laying hens showed its slower elimination mainly *via* bile, with the bioaccumulation in the liver, muscle, and eggs.[37] These results clearly show that the metabolism of a pesticide is one important factor in controlling its bioaccumulation. When the compartment model, where the pesticide input to the liver is distributed to each organ *via* blood flow and eliminated in eggs and excreta, was used in the PBPK analysis, it described more pesticide residues in the fat of broilers than of laying hens.[38,39] This difference indicates the importance of the excretory route to eggs.

The potential bioaccumulation of a pesticide can be evaluated by various methods. EFSA has adopted the simple equation, $AR=(\alpha * FIR)/k_2$,[1] where FIR is a food intake rate relative to the body weight of an avian species to be assessed. The absorbed fraction of the ingested pesticide (α) and the elimination rate constant (k_2) can be obtained in the metabolism study. Alternatively, the structure–activity relationship approach is convenient for estimating the AR value of a pesticide, and the moderate correlation of AR with log K_{ow} (r=*ca.* 0.5) was reported for poultry and small birds.[40] Many kinds of empirical and theoretical models have been developed to estimate the terrestrial bioaccumulation of organic chemicals, including pesticides, using their physico-chemical properties and environmental fate data, as reviewed by Gobas *et al.*[41] The partition coefficient of a pesticide between *n*-octanol and water (K_{ow}) or air (K_{OA}) is the primary factor for determining the AR value, which has been confirmed recently in the trophic magnification (TM) of persistent organic pollutants (POPs) by stable isotope analysis.[42] The tropic positions (TP) of primary producers, prey, and an apex predator in the Canadian urbanized region were estimated from the fraction of ^{15}N in each, as determined by MS, and the ^{15}N isotopic enrichment factor constant. The TM factors were determined from the correlation of TP with the lipid equivalent residue concentrations of POPs in each species. The TM factor was found to be well proportional to the log K_{ow} and log K_{OA} values of each POP (r=–0.99), showing the potential biomagnification of an organic chemical having a log K_{ow} (K_{OA}) of >4–6.

Table 2. Typical metabolic reactions of pesticides in birds

Oxidation		Reduction		Hydrolysis (Bond cleavage)	
O1	Alkyl oxidation/*O* (*N*)-dealkylation	R1	Reductive dehalogenation	H1	Carboxyl ester
O2	Aryl hydroxylation	R2	Dehydrohalogenation	H2	Amide, imide, carbamate, urea
O3	Epoxidation	R3	Multiple bond (–=N–, –C=C–, C=O)	H3	(Oxime, thio) Ether
O4	*S*-oxidation	R4	Nitro group	H4	C(N)–C(N, S) bond
O5	Desulfuration (P=S, C–O_3H)	R5	*S*-reduction	H5	Phosphoryl (sulfonyl) ester
Conjugation				H6	Dehalogenation/denitration
C1	Glucuronidation	C5	Acetylation	Miscellaneous	
C2	Sulfation	C6	Methylation	M1	Rearrangement
C3	Glutathione*	C7	Other natural products (fatty acids, cholesterol *etc.*)	M2	Cyclization
C4	Amino acid			M3	Hydration/dehydration

*R-X+GSH → R-SG → R-Cys-Gly → R-Cys → R-(*N*-acetyl-Cys). R, alkyl or aryl; GSH, glutathione; Cys, cysteine; Gly: glycine.

2. Avian metabolic enzymes

The metabolic reactions in birds, phase I (oxidation, reduction, and bond cleavage) and phase II (conjugations), as listed in Table 2, are basically similar to those in mammals.[43] In general, the microsomal fraction contains CYPs and UDPGTs, while glutathione-*S*-transferases (GSTs) and SULTs are localized in the cytosolic fraction, and esterases are distributed in both fractions.

2.1. Oxidases and reductases

CYPs, which catalyze the oxidation of their various moieties, are among the most important enzymes in the metabolism of pesticides. The total amount of CYPs in the avian liver ranges from 0.1 to 0.4 nmol/mg protein, which is lower than that of mammals (0.3–1.5 nmol/mg protein)[43–46]; therefore, the hepatic CYP activity of birds is generally comparable to or slightly lower than that of mammals and is highly dependent on the substrate.[26,43,47] Both the total amount and activity of CYPs also depend on avian species and vary by age, sex, and strain. The age-dependent increase in hepatic enzyme activity was reported for ethoxyresorufin *O*-deethylase (EROD; CYP1A) and pentoxyresorufin *O*-dependenthylase (PROD; CYP2B) in turkey poults (9–65 days old),[48] and for mephobarbital *N*-demethylase (CYP2B) in chicken embryos (15–18 days old) and chicks (4–9 days old).[49] A slight decrease in the aldrin epoxidase (AEase) activity with development was observed in the liver of the Japanese quail (7–35 days old),[50] while such a change was insignificant for *o*-nitroanisole *O*-demethylase in the chicken.[49] Using the liver of broiler chickens (1–56 days old), Hu[51] demonstrated that each activity of CYPs (1A, 2H, 2C, 2D, and 3A) is highest just after hatching and, because of the intense lipid metabolism in embryos and thereafter, gradually decreases with development. Less than a twofold difference by sex was reported for aminopyrine demethylase (ADase) activity in the liver and kidneys of chicken and geese, but the hepatic activity differs by a factor of *ca*. 4 between two strains of chicken.[27] Smaller differences by both sex and strain were reported for the hepatic amount and activity of AEase in the Japanese quail.[50] The post-mitochondrial subcellular fraction (S9) of the chicken liver exhibited 2–50 times sex differences (male >> female) in the oxidative degradation of eight OPs.[52]

The hepatic ADase activity was 1.1–4.8-fold higher than the renal activity in the chicken, turkey, duck, and goose,[27] indicating the liver's greater importance in pesticide metabolism. The alimentary tract may play a role in the metabolism of pesticides when considering its organ weight relative to the liver. Both the amount of CYP and the enzyme activities of EROD and AEase are comparable between the liver and duodenum in each of four avian species, and the weight ratio of the duodenum to the liver ranges from 0.3 to 0.6.[44,53] The ADase activity (nmol/hr/mg dry tissue) in the alimentary tract of chicks decreased in the order of duodenum (8)>mid-intestine (6)>rectum (2)>crop (2).[27] The activities of EROD and PROD in the small intestine were high, but less than those reported in the liver of the pigeon.[54] Incidentally, the feeding habit controls the CYP activity. Fossi *et al.*[25] measured the hepatic AEase activity in four europhagic and three stenophagic avian species collected from the Italian field. They assigned a score to each of six food categories by the percentage of each relative to the total food of birds, and the calculated total score for each avian species was used as an omnivorous index. The AEase activity was directly proportional to this index (r=0.7). By taking account of the EROD and PROD activities, it was found that the narrower the diet range of an avian species, the lower its hepatic CYP activity.[25,54]

Enzyme induction is another issue to be considered in either the metabolism or toxicity of pesticides. CYPs can be induced in birds—for example, by β-naphthoflavone, phenobarbital, and 3-methylcholanthrene[55]—as reported in mammals. Seven-day dietary exposure to dieldrin at 5 ppm increased the hepatic AEase activity in the Japanese quail twofold.[50] No induction of hepatic aniline hydroxylase (AHase) by DDT at 100 ppm was observed in this species, while DDE activity increased by a factor of 2–3.[56] Neither ADase nor AHase was induced by DDT, with their activity decreasing in the White Leghorn hen.[57] In the case of seven days of dietary exposure to four pyrethroids at 2000 ppm, the organ-dependent effects on the activity of AHase, AEase, and EROD as well as the total amount of CYPs were reported in the Japanese quail.[53] Insignificant effects were

observed in the liver, but the pyrethroids weakly induced these enzymes in the duodenum. Three dicarboximide fungicides differently induced hepatic CYP activity in this species.[53] Through seven days of dietary exposure to iprodione and vinclozolin at 2000 ppm, the activities of AHase, AEase, and EROD increased by a factor of 2–3, while procymidone only induced EROD. The induction of CYPs in the liver of the northern bobwhite quail was examined by the oral administration of several pesticides at 400 ppm once a day for three days.[58] Western blot analysis using several antibodies to rat CYPs showed that both vinclozolin and propiconazole increased three- to sixfold the amounts of CYP 1A1/2, 3A, and 4A1, and newly induced 2A1 and 2C11. Interestingly, azole fungicides exhibit a compound-specific effect on hepatic CYPs. Imazalil at 1000 ppm was a weak inducer of AHase in the northern bobwhite quail,[59] while the activity of AEase, AHase, and EROD increased up to sixteen-fold with three weeks of dietary exposure to prochloraz at 750 ppm in four avian species.[44] In the Japanese quail, ten azole fungicides caused the dual effect of induction and inhibition with the dietary exposure.[60] For example, propiconazole increased the hepatic activity of both AHase and EROD, fenarimol only decreased the former, and triadimefon only increased the former.

The presence of many avian CYP families is going to become clear by the analysis of the gene sequences derived from the DNA clones of, for example, CYP1A, 2H, 2E, and 3A in the chicken.[45] More than 20 CYP1-3 genes are known in the chicken, zebra finch, and turkey, and the dominant role of the chicken CYP2C45 is supposed to be for xenobiotic metabolism.[61] However, information relevant to its family's participation in pesticide metabolism seems scarce. Rawal and Coulombe[62] examined the metabolism of aflatoxin B_1 (AFB_1) in the hepatic microsomes of the toxin-susceptible turkey, using the monoclonal antisera against specific CYPs. It was found that the formation of the toxic metabolite, *exo*-8,9-epoxide of AFB_1 (AFBO), was mainly catalyzed by CYP1A5 with the minimal contribution of CYP3A37 (<2%). Furthermore, as the detoxification pathways, CYP1A5 participated in the hydroxylation of the terminal furan ring of AFB_1, while CYP3A37 oxidized its cyclopentenone ring. Recently, more CYP families in the liver were found to be involved in AFB_1 metabolism in avian species, the turkey and duck (1A1/2, 2A6, 3A4) and the chicken and quail (1A1, 2A6).[63] A similar approach to pesticide metabolism should be useful for understanding the role of each CYP in detoxification and/or bioactivation. The regioselectivity of avian CYPs against pesticides has been studied, together with its species differences, by using hepatic microsomes. The isopropyl methine and thiophosphoryl groups of diazinon were almost equally oxidized in the duck and turkey, and the methyl group of the pyrimidinyl ring was less susceptible to oxidation, while the oxon derivative was the main metabolite in the chicken.[64] The aromatic carbons of warfarin were more favorably hydoxylated in four avian species than its methylene carbon, and the extent of oxidation decreased in the order of 4′-OH >6-OH >7-/8-OH.[65] The *S*-oxidation of aldicarb to its sulfoxide was the main reaction in the chicken, with the corresponding sulfone as a minor metabolite.[66]

Information on avian reductases participating in the metabolism of pesticides is very limited. In the liver and kidney homogenates of the chicken and the English sparrow, flavoprotein nitroreductases catalyzed the reduction of the nitro groups of parathion and EPN with flavin adenine dinucleotide as a cofactor.[67] Comparable enzyme activity was observed between the two organs of each species, but with activity in the chicken twice as high as that in the sparrow. No induction of aromatic nitroreductases was reported in Japanese quail fed DDT or DDE at 100 ppm for three weeks, using *p*-nitrobenzoic acid as a substrate.[56] Through the metabolism of AFB_1 in the turkey, the aldehyde reductase played a detoxification role, and its presence in the hepatic cytosol was confirmed by western blot analysis.[48] The stereo-specific keto reduction of warfarin (*S*>*R*) to alcohol in the *S*-configuration proceeded in the hepatic cytosol of the chicken.[68] The complete inhibition of the reduction by menadione suggested the involvement of aldehyde oxidases.

2.2. *Esterases*

Esterases have been classified conveniently from the viewpoint of their hydrolyzing ability and inhibitors.[69] A-esterases such as phosphotriesterases (PTEs) can hydrolyze aromatic esters and are not inhibited by OPs. The structure of the PTE active site is not known, except that of *Pseudomonas diminuta*, where two zinc cations chelated by at least four histidine residues catalytically hydrolyze OPs. Either the rapid dephosphorylation of the esterase-OP complex[70] or no formation of such a tight complex as described above most likely accounts for the lack of inhibition by OPs. In contrast, B-esterases are inhibited by OPs and carbamates, such as ChEs and CaEs, whose active sites are the serine residue in the conserved motif.[69]

The hydrolyzing activity against OPs was examined for PTEs in the blood plasma of 14 avian species.[18,71] Paraoxon could be hydrolyzed only by the PTEs in the mute swan and Canada goose, with their activities lower than those of 11 mammalian species by two to three orders of magnitude. In the case of the pirimiphos-methyl oxon as a substrate, four avian species had low hydrolytic activity with inter-species differences similar to those above. Much lower PTE activity should result in the higher susceptibility of birds to toxic oxons than that of mammals.[72] The PTE activities in the blood plasma and microsomal and cytosolic fractions of the liver and brain were compared between chickens and rabbits, using (*RS*) *O*-hexyl *O*-2,5-dichlorophenyl phosphoramidate (HDCP) as a model substrate.[73] The stereospecificity observed for the PTE (*S*>*R*) in rabbit plasma was lost in that of the chicken, with its activity much lower by two orders of magnitude. The stereo-specific activity of the hepatic microsomes (*S*>*R*) of the chicken was about fourfold higher than that in the cytosols (*S*<*R*). The brain fractions showed comparable activity in both species, but lower than that of the liver by a factor of 2–10 with the same specificity. The same research group next examined the reaction kinetics of this PTE in chicken

serum albumin.[74] Both HDCP and *p*-nitrophenyl butyrate were competitively hydrolyzed by this enzyme, which was not inhibited by Ca^{2+} and EDTA. Therefore, the reaction mechanism of this enzyme would be different from that of the usual PTEs, and it was supposed to be similar to that of ChEs.

The B-esterase activity of birds and mammals was comparable when procaine[26] and phenyl acetate (PhA)[71] were used as substrates. B-esterases such as CaEs and ChEs in the liver, kidney, and intestine of the duck,[75] chicken,[76] and quail[77] were separated into many isoforms, using polyacrylamide gel electrophoresis (PAGE). Age-dependent enzyme activity was reported especially for CaEs in the liver and plasma of the duck and chicken, with their maxima after hatching.[75,78] Similarly to CYPs, B-esterase activity depends on either avian body weight or feeding habit. Bush *et al.*[79] reported in 55 avian species that the hepatic B-esterase activity against PhA and tricelin qualitatively correlates with birds' body weight and diet range. By measuring the brain AChE and plasma CaE activity of seven avian species, Fossi *et al.*[24] showed good correlation between the brain AChE and plasma CaE activity with not only avian body weight (r=–0.77 and –0.87) but also the omnivorous index (r=0.55 and 0.67), respectively. However, no correlation was observed for either CaE in the hepatic microsomes or butyryl ChE (BChE) in the plasma. In contrast, the total activity of AChE and BChE in plasma highly correlated with the body weight of 20 European raptors (r=–0.71), and the dominant ChE differed among the avian family.[80] The involvement of B-esterases has been indirectly demonstrated in the *in vitro* metabolism of some pesticides by using an inhibitor. The *in vitro* metabolism of phenmedipham in the blood plasma of the chicken[81] and that of *cis*-cypermethrin in the hepatic microsomes of the Japanese quail[82] were greatly suppressed by the addition of OPs or carbaryl.

2.3. *Glutathione S-transferases* (*GST*)

GSTs, the homo or heterodimers with each subunit having a molecular weight of 24–28 kDa, catalyze the reaction of glutathione at the electrophilic site of a pesticide and its metabolites,[83] as reported for the *O*-demethylation of many OP pesticides.[52,84,85] The intact glutathione conjugate has been detected rarely in the avian metabolism,[86,87] due to the successive metabolism by peptidases and *N*-acetyltransferases finally to form a mercapturic acid conjugate.[83] When the herbicide propachlor was orally administered to hens, the cysteine and mercapturic acid conjugated *via* the reaction of glutathione at the chloromethyl carbon were detected as the main metabolites in the excreta.[88]

The GST activity greatly varies by not only a substrate but also a species.[89] A comparative study of the GST activity in the hepatic cytosolic fractions of the northern bobwhite quail, rainbow trout, and six mammals was conducted against six substrates.[43] Quail showed the lower-to-lowest GST activity of any substrate among the eight species, and 1-chloro-2,4-dinitrobenzene (CDNB) was the best substrate. *In vitro* metabolism of three OPs by using liver homogenates showed less *O*-demethylation in the chicken than in mammals.[52] Hepatic GST activity against 1,2-dichloro-4-nitrobenzene (DCNB) was reported to be much lower in the turkey than in the rat, but comparable activity against CDNB was observed in both species.[90] In the kidney of the Japanese quail, the GST activity against DCNB was comparable to the hepatic activity.[91]

GSTs can be conveniently classified into at least seven families in mammals, depending on the physico-chemical, structural, and immunological properties.[92] Nine GSTs were isolated from the liver of chicks by using the glutathione affinity chromatography and chromato-focusing, and the molecular weight of each subunit was determined to be 24–27 kDa by SDS-PAGE.[93] Furthermore, at least six α-GSTs and three μ-GSTs were identified by the analysis of the amino acid sequences and LC-MS/MS.[94] In the case of the turkey, the hepatic cytosolic GSTs were classified into three α-GSTs and one σ-GST by western blot analysis using the respective antibodies against rat and chicken GSTs.[90] Another research group reported that the hepatic GSTs of the turkey are heterodimers with optimal activity at a pH of 7.5 and 50°C against CDNB.[95] Similar enzymology, but with homodimers, were reported for the hepatic GSTs of the Japanese quail.[96,97] Incidentally, AFB_1 is more toxic to birds than mammals due to their lack of the ability to metabolize the toxic metabolite AFBO by GST.[62,63] The recombinant α- and μ-GSTs prepared by cloning the corresponding genes of the turkey well reproduced their activity against CDNB and DCNB.[98] While they could also conjugate AFBO at a comparable reaction rate against DCNB, no activity was observed for the intact GSTs in the hepatic cytosolic fraction. Therefore, the GSTs metabolizing AFBO may be silenced by some regulatory mechanism in birds.

2.4. *Other enzymes relevant to conjugation*

UDPGTs and SULTs catalyze the glucuronidation and sulfation mainly at the hydroxyl oxygen by using uridine 5′-diphosphoglucuronic acid (UDPGA) and phosphoadenyl phosphosulfate (PAPS) as co-factors, respectively.[83] Several pesticides such as carbaryl,[99] fenitrothion,[140] bifenazate,[140] and prothioconazole[140] instead undergo conjugation at the nitrogen and sulfur atoms of their metabolites. The hepatic activity of UDPGT and SULT highly depend on both the substrate and species.[26,43] As compared with nine mammalian species, four avian species showed comparable or lower UDPGT activity levels against 4-nitrophenol, and lower SULT activity levels were mostly observed against 2-naphthol. In the comparative *in vitro* metabolism of cypermethrin, using hepatic S9 fractions, the dominant conjugation was the glucuronidation of the hydroxylated insecticide in rats, while the acyl glucuronides of both 3-phenoxybenzoic acid (PBacid) and the acid moiety of the insecticide were mainly formed in the Japanese quail, because of the rapid ester hydrolysis.[82]

Among nine avian species, the hepatic activity of these enzymes varies by one to two orders of magnitude.[27,100] Furthermore, which enzyme dominantly contributes to the conjugation depends on the avian species. For example, SULT is more

involved in the conjugation of phenols than UDPGT in the livers of chickens and ducks,[27,101] and the opposite is observed in those of turkeys and Japanese quail.[27,102] Either age- or organ-dependent activity was reported for these enzymes. The hepatic enzyme activity, especially SULT, in the chicken was highest just after hatching, and lower thereafter.[101] Their activity per dry weight of tissue in chicks decreased in the order of duodenum ≒liver>mid-intestine>rectum.[27] Incidentally, through a detailed sequence analysis of the UDPGT genes in 43 avian species, Kawai *et al.*[100,103] showed the presence of UDPGT1E and UDPGT2 genes, which are further classified into six and three groups, respectively; feeding habits may affect not only the number of UDPGT1E genes but also the hepatic enzyme activity (carnivorous sp.<omnivorous and herbivorous sp.). A search of the chicken expressed sequence tag database has identified two cDNA clones that represent SULT subfamilies 1B and 1C.[104] The recombinant SULTs, having histidine residue as an active site, could catalyze the sulfation of various phenols.

Carboxylic acids, irrespective of pesticides, their metabolites, or natural products, react with coenzyme A (CoA) to form a corresponding thioester.[83,105] Acyltransferases (AcTs) catalyze the reaction of this thioester with amino and hydroxy derivatives to form amides and esters, respectively, but the enzymology of avian AcTs has not been extensively investigated. Although the hepatic cytosolic fraction of the rabbit possesses the high activity of acetyltransferases against many substrates, much lower activity in birds has been reported with the exception of some substrates, such as 2-aminofluorene.[26,43] The nitro groups of parathion and fenitrothion were reduced and then *N*-acetylated in the liver and eggs.[140] PBacid, the major metabolite of several pyrethroids, is conjugated with glycine, glycylvaline, and *N*-acetylornithine in mallard ducks and chickens,[106,107] and the conjugation profiles are highly dependent on the avian species.[108] Another conjugation of the long-chain fatty acid with the hydroxylated metabolite is reported for tebufenozide and bifenthrin.[140]

3. Metabolism in birds

Information on the metabolism of many industrial chemicals, drugs, and pesticides by various avian species was accumulated up to the last quarter of the 20th century.[108] The requirement for registering the poultry metabolism study by using a radiolabeled pesticide,[5] in parallel with recent progress in LC(GC)-MS^n, has provided more detailed information regarding avian metabolism. The metabolic pathway of a pesticide is generally examined through the chemical identification of metabolites by co-chromatographies with synthetic standards and/or instrumental analyses. In the case of polar conjugates, the aglycon released by chemical or enzymatic hydrolysis is frequently subjected to identification.

3.1. In vitro *metabolism*

In vitro metabolism is easily conducted and useful for grasping the major metabolism profiles in birds, as listed in Table 3. The S9 fraction, microsomes, and cytosols are prepared by the differential centrifugation of the homogenate of the liver, kidney, or intestine in the established manners.[82,91,102] The addition of enzyme cofactors such as NADPH, glutathione, UDPGA, and PAPS is sometimes necessary to facilitate the metabolic reactions. Instead of these subcellular fractions, tissue slices[67,111] and hepatocytes[117,121] can be alternatively used (*ex vivo*). The metabolism study is usually conducted at 37°C and/or 42°C, which are the typical body temperatures of mammals and birds, respectively. The oxidation of alkyl and/or aryl moieties is frequently observed, as well as the hydrolysis of the ester and amide linkages. Reduction occurs less than oxidation, for example, for the chlorinated alkyl moieties of lindane[109] and DDT,[111] the nitro group of parathion,[67] and the ketone of warfarin.[68] *O*-Glucuronidation of the phenolic oxygen often proceeds in the metabolism, as reported for cypermethrin,[82] carbaryl,[91] NMC,[102] and methoxychlor,[112] while conjugations with a sulfate or glutathione are less observed. In the case of PCPMs, the FAB-MS measurement succeeded in directly identifying their glutathione conjugates.[86] The concomitant formation of *S*-methyl glutathione with *O*-demethylation of tetrachlorvinphos was observed by incubation in the hepatic cytosolic fractions with glutathione, showing that GST catalyzes this reaction.[87]

3.2. In vivo *metabolism*

3.2.1. Route of administration

The oral administration of a pesticide is generally conducted by gavage or intubation, and feeding a diet treated with pesticide is alternatively conducted for a longer period of study. Intravenous administration causes such a rapid uptake of pesticide into the body that the basic profiles can be conveniently obtained for its distribution, metabolism, and elimination. The effect of an administration route on the metabolism of pesticides has not been examined so extensively. When DDT was orally administered at the same dosage to the northern bobwhite quail by feeding or intubation, the latter method increased the absorbed amount of DDT through the gut wall, but the metabolism in the liver was not changed by the administration method.[124] Although the metabolic profiles of [benzyl-^{14}C]cypermethrin in the Japanese quail were almost independent of the administration method, by gavage or intraperitoneal injection, lesser elimination of ^{14}C to excreta was observed in the latter, probably due to the rapid distribution of ^{14}C to each organ, especially adipose tissues.[125] Kinetic analysis has been applied to the metabolism study of permethrin[126] and deltamethrin[127] in broilers by different administration methods—intubation into the crop and intraperitoneal injection. In intra-crop application, the rapid absorption of pyrethroids *via* the digestive system occurred, and the slower elimination rate with the mean absorption time longer than those with injection indicated continuous absorption from the gastrointestinal tract during elimination. The low bioavailability of these pyrethroids (11–22%) was estimated by comparing their concentration curves in serum after the two types of administration.

Table 3. *In vitro* metabolism of pesticides in birds

Pesticide[a)]	Sp.[b)]	Organ[c)]	°C	Pro[d)]	Sub[e)]	Cofactor[f)]	MR[g)]	Ref.
Lindane	CH	L (c)	37.5	na	3.8†	± GSH	R2	109
Aldrin	CH	L (S9)	41	na	na	NADPH	O3	110
DDT	FP	L (ts)	41.5	0.1*	11	± GSH	R1/2	111
Methoxychlor	JQ	L (ts)	39	na*	5	none	O1, C1	112
PCPMs	CH	L (m)	37	62	2.5	GSH	C3	86
Carbaryl	JQ	L,K (m,c,S9)	37	4	1	NADPH, GSH	O1, H2, C1/2/3	91
Aldicarb	CH	L (m)	rt	3	50	NADPH	O4	66
Phenmedipham	CH	B	37	na	82	none	H2	81
Trichlorfon	CH	L (c)	37.5	na	73	none	C3	113
Tetrachlorvinphos	CH	L (c)	37.5	na	0.3†	GSH	R2/3, H5, C3	87
Diazinon	TU	L (ts)	37	0.4*	66	NADPH	O1/5, M3	64
Parathion	CH	L,K (ts)	37	10*	0.5†	NADPH	R4	67
Fenitrothion	CH	L (m,c)	37	na	70	NADPH, GSH	O1/5, H5, C3	85
NMC	JQ	L (m,c)	37	10	0.5	UDPGA, PAPS	C1/2	102
EPN	CH	L (m)	37	0.5–5	0.5†	NADPH	O5, H5	114
Chlortoluron	JQ	L,K (S9)	37	4	1	NADPH, GSH	O1	91
Diflubenzuron	CH	L (m)	37	3–5	21	NADPH	O2, H2	115
Cypermethrin	JQ	L,I (S9),B	42	na	10	NADPH,UDPGA	O1/2, H1, C1	82
Deltamethrin	CH	L (m,c)	37	0.2*	6.5	± NADPH	O1/2, R3, H1	116
Fenvalerate	JQ	L (h)	42	1‡	1.8	none	O1/2, H1, M3	117
PBacid	CH	L,K,I (m)	42	0.1*	0.3†	NADPH	O2, H3	118,119
Fenproximate	JQ	L (S9)	25	na	0.1†	NADPH	O1/2, M1	120
Kresoxim-methyl	CH	L (h)	37	2–5‡	0.1†	none	O1/2, H1/3	121
Atrazine	CH	L (c)	37.5	na	0.8†	GSH	O1, H6	122
Terbutryn	CH	L (m, S9)	37	1–5	0.1	± NADPH, GSH	O1, H6**	123
Warfarine	CH	L (m,c)	41	1	0.4†	NADPH	O2, R3	68

[a)] PCPMs, pentachlorophenyl methyl sulfoxide and sulfone; NMC, 3-methyl-4-nitrophenol (metabolite of fenitrothion); PBacid, 3-phenoxybenzoic acid (metabolite of several pyrethroids). [b)] Tested species. CH, chicken; FP, feral pigeon; JQ, Japanese quail; TU, turkey. [c)] L, liver; K, kidney; I, intestine; B, blood plasma. The words in the parentheses mean the microsomal (m), cytosolic (c), post-mitochondrial (S9) fractions, hepatocytes (h), and tissue slices or homogenates (ts), respectively. [d)] Protein concentration of each fraction in mg/ml; *, in g tissue/ml; ‡, in 10^6 cells/ml. [e)] Pesticide concentration in μM. †, in mM. [f)] NADPH, nicotinamide adenine dinucleotide phosphate; UDPGA, uridine 5′-diphosphoglucuronic acid; GSH, reduced glutathione; PAPS, phosphoadenosyl phosphosulfate. [g)] Metabolic reactions (see Table 2); **, hydrolytic dethiomethylation. na, not available. r.t., room temperature.

The dermal route becomes important when birds are directly exposed to pesticides applied aerially. Abou-Donia[128,129)] compared the fate of [Ph-^{14}C]leptophos in laying hens with a single administration either orally by gavage or dermally to each side of the combs at the same dosage. The two-phase elimination profiles to excreta were observed after administration by both routes, but with the fourfold faster elimination of ^{14}C in the first phase by gavage. Neither the ^{14}C distribution in tissues nor the metabolic profiles were significantly changed by the administration route. The same author's group has further compared the fate of [Ph-^{14}C]EPN in hens with a single administration either orally by gavage or topically daily to skin at the bird's neck for 10 days at similar dosages.[130,131)] Most of the dose (64–74%) was excreted by both administration types with the major ^{14}C residues present in the liver, kidney, and bile. Although the same metabolites were detected, the radioactivity in both non-conjugated and water-soluble fractions by the extraction of the excreta gradually increased as time passed after the single oral administration. Since the corresponding ^{14}C in the topical administration decreased with time, the hydrolysis of conjugated or bound ^{14}C-releasing aglycons was most likely to proceed in the gastrointestinal tract. These results show that both the administration method and route change the profiles of uptake, distribution, and excretion, but the metabolic pathway is basically the same.

3.2.2. Species differences

In the metabolism of kresoxim-methyl using the hepatocytes, though *ex vivo*, the phenoxy moiety was favorably hydroxylated at the 4-position in the chicken, while the 2-methyl group was the main site of hydroxylation in three mammalian species with more cleavage of the ester and ether linkages.[121)] Sulfentrazone *via* oral administration mainly underwent the successive oxidation of the methyl group attached to the hetero ring in rats, goats, and hens, while the reduction of the C=N bond proceeded only in rats.[132)] In the case of orally administered thiabendazole, the same metabolites, formed *via* hydroxylation at the 5-position of the benzimidazole moiety followed by sulfa-

tion, were detected in the excreta of hens and goats, but more so in goats.[133] The main metabolism of fenitrothion in birds is the cleavage of the P-Oaryl linkage, followed by the oxidation of the aryl methyl group and/or conjugation with sulfate on the resulting phenol, and the sulfation markedly proceeded in the Japanese quail, as compared with the hen.[85] Furthermore, strain differences in the metabolic profiles were reported for [Ph-^{14}C]-diflubenzuron between White Leghorn hens (WL) and Rhode Island Red/Barred Plymouth Rock (RIR/BPR) chickens.[115,134] The faster uptake and elimination of ^{14}C were observed in the RIR/BPR strain than in the WL strain, and the major elimination route was different between RIR/BPR (excreta) and WL (eggs). The same metabolites were detected in both strains but with different profiles of the bond cleavage in the benzoylurea bridge connecting the two phenyl groups.

Species differences in conjugations have been extensively studied for pyrethroids and their major metabolite, PBacid. The analysis of metabolites in the excreta after the oral administration of cypermethrin showed different metabolic profiles among the Japanese quail, rat, and mouse.[125] Although the total elimination was similar (84–93% of the dose), both the ester cleavage and oxidation at the 4′-position of the α-cyano-3-phenoxybenzyl moiety proceeded in the decreasing order of rat>mouse>quail. The main reactions of the metabolites, PBacid and its 4′-OH derivative, were quite different among the species, that is, the sulfation of 4′-OH-PBacid (rat); the conjugation of PBacid with taurine (mouse); and the specific conjugation of PBacid with ornithine, *N*-acetylornithine, serine, and glycylvaline (quail). Extensive metabolism studies of PBacid have shown that the main reactions *via* its oral administration are its conjugation with glycine (dog), glutamic acid (cow), glycylvaline (mallard duck), and ornithine (chicken).[106,107] Judging from these limited metabolic data, the main metabolic pathways of pesticides are quite different in birds and mammals, especially for the conjugation of metabolites. Furthermore, the relative contribution of each metabolic reaction seems different, even among birds and their strains.

3.2.3. Isomerism

The biological efficacy and/or toxicity of a pesticide frequently depend on its isomerism, at least in part, due to the stereoselective metabolism. After the oral administration of [^{14}C]-permethrin to laying hens, the *trans*-isomers more rapidly dissipated from the blood due to the faster hydrolysis of ester than of *cis*-isomers.[135] As a result, more of the hydroxylated intact ester and its conjugates were formed from the *cis*-isomers, though almost the same ^{14}C residues between the isomers were detected in the excreta. The hydroxylation of the *gem*-methyl group in the acid moiety proceeded favorably at the *cis*-position to the carboxyl group, and a similar profile was reported for cypermethrin.[136] The elimination kinetics of diniconazole (*E*-isomerism) by single oral administration to the Japanese quail was analyzed in the blood, heart, liver, and kidney.[137] Rapid elimination, with a half-life of 2–5 hr, was observed in any organ for the *S*-isomer, while the *R*-isomer dissipated 16–28 times slower in the liver and kidney. The enantio-enrichment of the (-)-*cis*- and (+)-*trans*-isomers in the liver and intestine was reported in the oral administration of chlordane to chickens, implying the presence of stereo-selective metabolism.[34] The involvement of CYP1A1/2 and CYP2B1 was indicated by inhibition experiments in the metabolism of metalaxyl by using the chicken hepatic LMH cells, and more rapid dissipation of the *S*-isomer was observed therein.[138] These results show the stereo-specific metabolism of pesticides by esterases and oxidases. In the case of methoxychlor, stereo-selective *O*-demethylation by oxidases was observed in the metabolism by using liver slices.[139] The resulting metabolite dominantly had an *S*-isomerism in the rat and mouse, while the opposite was observed in the Japanese quail and trout. Although information on the stereo-selective reduction of pesticides is very limited, the degradation of HCH in the hepatic cytosolic fraction of laying hens is highly dependent on its isomerism.[109] The γ-isomer (lindane) rapidly underwent dehydrochlorination, but the reaction was much slower for α- and δ-isomers, and no metabolism of β-isomers was observed.

3.2.4. Metabolic profiles

The report and evaluation by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and Pesticide Specifications (JMPS), available for many pesticides, are useful for understanding their metabolism, mainly in laying hens.[140] Based on these information and our literature survey, the metabolic profiles of representative pesticides in each chemical class are listed in Table 4.

Organochlorine (*OC*) *pesticides*

Cyclodiene insecticides are metabolized in birds generally by oxidation. The alkenyl bond of aldrin[141] and the chlorinated cyclopentyl ring of chlordane[34] are rapidly epoxidized, resulting in their short residence in the chicken body. In the case of endosulfan, the primary metabolic routes in the laying hen are *S*-oxidation and the hydrolytic opening of the 2-oxo-1,3,2-dioxathiolane ring followed by the ether formation from the resulting diol.[140] The sulfate derivative was the main metabolite in the eggs, muscle, and fat of laying hens, and its persistent character causes a toxic concern. The reductive dechlorination and/or dehydrochlorination are the main reactions of DDT[142,143] and lindane, respectively.[140] The hydrophobic character of these insecticides and their metabolites results in their high residues, especially in the fat. Having an analogous chemical structure to DDT, methoxychlor undergoes *O*-demethylation, followed by glucuronidation.[144] The polychlorinated benzene derivatives used as fungicides and herbicides show the different types of metabolism in laying hens. The dechlorination of chlorothalonil proceeds hydrolytically,[140] and the replacement of chlorine atoms by glutathione, followed by the successive degradation to form the corresponding cysteine conjugate, was reported for dichlobenil and dicloran.[140] Both dicamba and quinclorac undergo *O*-demethylation and/or glucuronidation instead of dechlorination.[140]

Organophosphorus (*OP*) *pesticides*

The sulfur atom in the thiophosphoryl moiety is oxidatively desulfurated, while the sulfide is successively oxidized *via* sulfoxide to sulfone, as reported for phorate and fenamiphos.[140] The

Table 4. *In vivo* metabolism of pesticides in birds

Pesticide[a)]	Period (day)[b)]	Metabolic profiles			Pesticide	Period (day)	Metabolic profiles		
		% dose[c)]	Reactions[d)]	Ref.			% dose	Reactions	Ref.
Lindane	4–6	44–63, 3–9, 2–4	R1	140	Carbaryl	7 (2)	98, 0.1, <0.1	O2, H2, C1/2/3	99, 140
DDT*†	1/2–3	65–66/5–16, —, —	R1/2	142, 143	Chlorpropham	7	83, <0.1, —	O1/2, H2, C1/2/5	140
Methoxychlor	14	77–92, —, —	O1, R1, C1	144	Indoxacarb	5	87–88, 0.3–0.4, <1	O1, H2/3/4, M2	140
Phorate	5	62–66, 0.7–1.5, <1	O4, H5, C7	140	Diflubenzuron	1	91, 0.8, —	O2, H2	140
Tetrachlorvinphos	7	74–77, <1, —	R1/3, H5, C3	141	Methoxyfenozide	7	84–93, <0.1, <0.1	O1/2, C1	140
Fenitrothion**	7/1	93–102, <0.2, —	O1/5, H5, C1/2/3	85	Fenamidone	14	>88, —, —	O2, H2/4, C1	140
EPN	21	94–100, —, —	O2/5, R4, H5	145	Pinoxaden	4	75, <0.1, <0.1	O1, H1/3	140
MCPA	7	99, 0.1, <0.1	C4	140	Cycloxidim	10	78, 0.3, 0.1	O4, H3, M1	140
Cypermethrin***	1	75–87, —, <2	O1/2, H1, C1/4	125	Clothianidin	3	95, 0.2, —	O1, H2/4, C5/7, M2	140
Fluvalinate	1–3	92–94, <0.4, —	O1, H1/3, C1/4	146–148	Kresoxim-methyl	6	72–83, <0.2, <0.1	O1/2/4, H1/3, C2	140
Bifenthrin	10	>90, <0.4, <0.8	O1, H1, C7, M2	140	Tolyfluanid	1–3	84, <0.1, <0.1	O1, H4, C4	140
Fluazifop-*p*-butyl	11	90–98, —, —	H1/3	140	Chlorfenapyr	7	78–94, —, —	O1/2, R1, H6	140
Isofetamid	14	103–116, <0.2, <0.1	O1/2, H2	140	Thiacloprid	3	75, <0.1, <0.7	O1/4, H2/4, C4/6, M3	140
Fluopicolide	14	82–95, 0.1, <0.2	O1/2, C2/3	140	Atrazine‡	7	—, —, —	O1, H6	149, 150
Benzovindiflupyr	14	88–92, 0.1, <0.1	O1/2, H4, C1/2	140	Propiconazole	8–16	94–103, <0.6, <0.2	O1, R3, H4/6, M3	140
Flubendiamide	14	62–66, 5–8, —	O1, C1, M2	140	Tiadimefon	3	—, —, —	O1, C1/2	140
Acetochlor	7	68–82, <0.5, <0.3	O1, H3, C3/5	140	Cyproconazole	3–4	94–97, 1–2, <1	O1/2, R3, H4, C2	140
Chlorantraniliprole	14	98, 3, —	O1, H2, M2	140	Fludioxonil	8	88–112, —, —	O1/2/4, H4, C1/2	140
Aldicarb	14 (2)	85, 5, —	O4, H2/3, M3	140	MAB_{1a}	7	64–69, <0.1, —	O1, C7	151

[a)] Tested species, laying hens. *, Japanese quail and feral pigeon; **, laying hens and Japanese quail; ***, Japanese quail. [b)] Oral administration once a day. '(2)' means a twice administration a day. †, intraperitoneal administration. ‡, feeding. [c)] Distribution (^{14}C) in excreta, eggs, and muscles; —, not available. [d)] Metabolic reactions listed in Table 2.

main reaction of glyphosate and glufosinate is the oxidation of the alkyl moiety (*β*-oxidation).[140] The alkyl group attached to an aromatic ring undergoes successive oxidation for fenitrothion[85] and tolclofos-methyl,[140] and regioselectively for diazinon.[140] Another typical reaction is the cleavage of either the P–OAr or P–OCH_3 linkage. The former reaction is catalyzed by esterases, while the latter proceeds *via* the conjugation with glutathione.[87] The resulting phenol is generally conjugated with glucuronic acid (GA) and sulfate, as reported for diazinon[140] and fenamiphos,[43] respectively, and both conjugates are formed from fenitrothion,[85] profenophos,[140] methyl parathion,[152] and EPN.[153] The cleavage of the P–OAr linkage was the dominant metabolic route of EPN in laying hens,[145] while *O*-demethylation and/or desulfuration of leptophos proceeded to a comparable extent of the P–OAr bond cleavage.[128] As a reductive metabolism, the conversion of the nitro to amino group is observed for parathion[140] and EPN.[145]

Acid and ester pesticides, including pyrethroids

β-oxidation to finally form the corresponding phenol is one of the metabolic pathways of the phenoxyacetic acid derivatives and their esters, as reported for 2,4-D,[154] while the cleavage of any ether linkage was not observed for fluazifop-*P*-butyl and haloxyfop.[140] The *bis* adduct of MCPA with ornithine was the major metabolite in the egg yolk and muscle of laying hens.[140] In the case of methoprene, the cholesteryl ester of its acid was detected in the chicken liver.[140] Many researchers have extensively examined the metabolism of pyrethroids, mainly in laying hens. One common reaction is the hydroxylation of the alkyl carbons in the acid moiety and/or at the 4′-position of the 3-phenoxybenzyl group, followed by conjugation with GA or sulfate, as reported for fenvalerate,[117] cypermethrin,[125] permethrin,[135] *lambda*-cyhalothrin,[140] fenpropathrin,[140] and deltamethrin.[155] In addition to esterases, the possible involvement of oxidases *via* the unstable *α*-hydroxybenzyl intermediate was proposed in the ester hydrolysis of cypermethrin.[82] Furthermore, the ether cleavage of PBacid was observed in the metabolism of fenpropathrin,[140] fluvalinate,[148] and deltamethrin.[156] The resulting metabolites generally undergo conjugation with GA, sulfate, and various amino acids, as described in Section 3.2.2. Several types of ornithine conjugates, including *bis* adduct, were additionally reported for the alcohol moieties of cypermethrin,[125] fluvalinate,[148] and deltamethrin.[156] Two types of a rare conjugation have been reported; one is the conjugation of the acid moiety of fluvalinate with bile acids such as taurochenodeoxycholic acid in the chicken metabolism.[146] Another one was reported for bifenthrin, of which, one of the *gem*-methyl groups was hydroxylated and then conjugated with oleic and palmitic acids.[140]

Amide, carbamate, and urea pesticides

These pesticides are basically metabolized *via* either the cleavage of the N(H)–CO bond, most probably catalyzed by CaEs or ChEs,[157,158] or the oxidation of aromatic and/or alkyl moieties, followed by conjugation with GA and/or sulfate, as listed in Table 4. The successive oxidation of the methyl group finally results in *O*- or *N*-demethylation. The electrophilic site of the pesticide is conjugated with glutathione, followed by enzymatic conversion to the corresponding cysteine and mercapturic acid conjugates, as observed for acetochlor[140] and propachlor.[88,159] The electrophilic conjugation with glutathione similarly proceeded at the 5-position of 1-naphthol, the main metabolite of carbaryl, and the 3-position of the benzoyl ring of fluopicolide.[140] In the case of methomyl, the main metabolite acetonitrile was conjugated with glutathione to finally form the *N*-cysteine derivative *via* rearrangement.[160] Other types of conjugation were the *N*-acetylation of the aniline metabolite of chlorpropham and the conjugation of the benzoic acid metabolite of tolyfluanid with glycine.[140] Although either the ring opening or intramolecular cyclization was observed for flubendiamide, indoxacarb, and chlorantriniliprole,[140] these reactions were likely to proceed abiotically.

Miscellaneous pesticides

Metabolism profiles similar to those above are generally reported for other pesticides (Table 4). In the case of chlorfenapyr, the debrominated derivative was detected as the minor metabolite in the liver of chickens.[140] In addition to the N–O bond cleavage and *S*-oxidation, cycloxydim underwent the Beckmann rearrangement to form the oxazolyl ring,[140] but this is an abiotic reaction. Many kinds of natural acids are conjugated with pesticides and their metabolites, for example glycine for thiacloprid,[140] glutamic acid for metaflumizone,[140] alanine and acetic acid for propiconazole,[140] pyruvic acid for clothianidin,[140] fatty acids (palmitic, stearic, and oleic) for tebufenozide,[140] oleic and seven other fatty acids for MAB_{1a},[151] and phosphoric acid for flusilazole.[140]

Conclusion

Oral uptake is generally the main route of exposure, based on the use pattern of pesticide formulations and avian feeding habits. In the first-tier avian risk assessment of pesticides, acute oral toxicity becomes preferable to short-term dietary toxicity based on its convenience and a more conservative estimation of toxicity. The acute oral toxicity of pesticide varies widely among avian species, depending on body size, feeding habits, and the activity of metabolic enzymes. Furthermore, several field studies have indicated the importance of dermal toxicity for more precise risk assessment of pesticides in the field. From these viewpoints, not only the species-sensitivity distribution approach to acute oral LD_{50} but also the contribution of acute dermal toxicity had better be considered for refining the avian risk assessment of pesticides exhibiting high toxicity.

In order to monitor the pesticide residues in poultry, if necessary, metabolism study is indispensable not only for identifying relevant metabolites but also for knowing the distribution of a pesticide and its metabolites in the edible commodities of laying hens. The PBPK approach also had better be taken at the same time to obtain kinetic data relevant to the distribution, metabolism, and elimination of the pesticide. Such data are very useful for evaluating the potential toxicity and/or bioaccumulation of

pesticide in birds *via* a dietary route. Since a variety of natural components in addition to GA, sulfate, and glutathione may be conjugated with a pesticide and its metabolites, a thorough identification of major polar metabolites should be conducted, especially for the edible portions of birds. Incidentally, the metabolic information in avian species other than chickens is very limited, except in cases in which higher species-specific toxicity is observed. It is not practical to conduct *in vivo* metabolism in many species; hence, *in vitro* and *ex vivo* studies should be conducted alternatively, using the subcellular fractions of metabolically important organs and/or hepatocytes. As with the accumulation of the relevant data above, the *in silico* approach to avian toxicity should be further refined by taking account of pesticide metabolism. Concerning metabolic enzymes, their enzymologies except CYPs have not been well examined yet. Not only gene analysis to find out the possible metabolic enzymes but also the classical biochemical approach to isolating and characterizing them is indispensable for knowing species differences in pesticide toxicity in more detail.

Electronic supplementary materials

The online version of this article contains supplementary material (Supplemental data), which is available at https://www.jstage.jst.go.jp/browse/jpestics/.

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