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Absorption, Distribution, and Excretion of [^{14}C]-3-Chloro-4-methylaniline Hydrochloride in Two Species of Birds Following a Single Oral Dose

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Ring-labeled [^{14}C]-3-chloro-4-methylaniline hydrochloride (250 μg per bird) was delivered to 21 red-winged blackbirds (*Agelaius phoeniceus*) and 21 dark-eyed juncos (*Junco hyemalis*) via oral gavage, and the distribution and excretion of radioactivity were determined at 15 and 30 min and 1, 4, 8, 12, and 24 h ($n = 3$ per time point). Direct measurement of radioactivity as well as measurement following combustion was accomplished using a liquid scintillation counter. Elimination from most tissues followed a two-compartment model, with very rapid elimination occurring between time 0 and 4 h and a much slower elimination phase occurring after that. The average half-life of elimination for the initial phase in most tissues examined was 0.16 h for juncos and 0.62 h for blackbirds. The average for the slower second phase of elimination was 3.4 h for juncos and 5.4 h for blackbirds. The radioactivity in blackbird kidney tissues did not change significantly for the duration of the test, pointing toward the kidney as a possible site of action for this important agricultural chemical.

KEYWORDS: 3-Chloro-4-methylaniline hydrochloride; CPTH; avicide; disposition; red-winged blackbirds; dark-eyed juncos

INTRODUCTION

Agricultural crops suffer damage and losses from many sources. Among sources of damage are those due to birds feeding on the crops. The extent of this damage can vary dramatically from location to location. In some cases, the damage from these birds can be so extensive as to result in loss of an entire crop. Such effects can be devastating to individual farmers (*1*). Some tools that are available to control or minimize damage to crops include hazing, fumigants, shooting, habitat modification, and use of lethal agents. One tool that has been used by the U.S. Department of Agriculture (USDA) is the lethal agent 3-chloro-4-methylaniline hydrochloride (3-chloro-*p*-toluidine hydrochloride, CPTH, DRC-1339, Starlicide). CPTH is used for the control of pest bird species that damage agricultural crops, present hazards to aircraft, or have the potential to threaten human health or safety. Extensive research has been performed to determine its toxicity to many target and nontarget bird species. It is a chemical agent that is a potent avicide to susceptible species and is capable of producing lethality following ingestion of as little as one grain of rice containing 2% CPTH (*2*). As part of an integrated pest management plan, use of CPTH has the potential to reduce crop damage caused by pest bird species.

Responsible use of any pesticide requires consideration of the potential effects to nontarget species. Although specific baiting techniques and methods are employed to minimize potential exposure to secondary and nontarget species (*3*), exposure is still possible. Possible exposures to nontarget species present a potential risk, which should be investigated. The toxic effects of CPTH to birds have been known since the 1960s (*4*, *5*). Since that time, its continued use has sparked extensive research of this chemical (*6*−*9*). A great deal is known about the acute toxicity of CPTH to various avian and mammalian species (*10*). Some investigations of the potential mode of action have been performed as well. These studies focused primarily on the pathological effects in exposed tissues and certain biochemical parameters such as blood pH (*7*, *9*, *11*). One thesis research project speculated which metabolites might be formed on the basis of likely metabolic pathways and then attempted to quantify these preselected metabolites in exposed bird species (*8*). Packed-column gas−liquid chromatography was used to provide quantitation of the selected metabolites. However, no known attempt has been made to identify potential intermediate or unknown metabolites or to elucidate a mechanistic mode of action for this powerful toxicant.

CPTH is a fairly selective pesticide with respect to the amount of chemical required to produce toxic effects in various species. It is somewhat common for differences in toxic response to exist between taxonomical classes or even orders. It is less common for large differences to exist within taxonomic families; CPTH

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possesses such a feature. An extensive review of the literature concerning CPTH toxicity has been performed (*10*). The data reviewed clearly demonstrate the differences in toxic response between varied avian families. Those whose acute oral LD_{50} values are >25 mg/kg could be referred to as resistant. Those possessing LD_{50} values of <25 mg/kg are characterized as sensitive to the toxic effects of CPTH. Understanding these differences could contribute to the more effective and safe use of this important compound.

MATERIALS AND METHODS

As a first step toward understanding these differences in toxic response, an aqueous solution (water from in-house filter system) containing ~9.25 μCi of [^{14}C]CPTH (51.32 mCi/mmol; 98.77% radiochemical purity; Wizard Laboratories Inc., West Sacramento, CA) was given orally to two species of birds: red-winged blackbirds (*Agelaius phoeniceus*), a CPTH sensitive species (LD_{50} = 1.8−3.2 mg/kg), and dark-eyed juncos (*Junco hyemalis*), a CPTH resistant species (LD_{50} = 162 mg/kg). The radioactive CPTH was isotopically diluted with non-radioactive CPTH (98.8% pure; Purina Mills) to produce the desired exposure level of ~250 μg per animal. The birds were housed in individual glass metabolism cages for a period of time ranging from 15 min to 24 h until they were euthanized by CO_2.

Test animals were trapped from wild populations in Colorado and housed indoors with free access to a standard wild bird seed mixture and water prior to testing. Test animals were held in quarantine for no less than 14 days prior to initiation of the test. For each time point, three birds were selected at random and given a dose of 250 μg of [^{14}C]CPTH in 100 μL of deionized water via oral gavage. The dosing solution was measured using a 100-μL Hamilton syringe and carefully transferred to a 1 cm^3 plastic syringe. Adequate headspace was left in the syringe to permit full delivery of the dose. The blackbirds had an average body weight of 70 g (SD = 3.5 g), whereas the juncos averaged 18 g (SD = 2.2 g). The resultant dose of CPTH for each species averaged 3.6 and 14 mg/kg for blackbirds and juncos, respectively. The birds were held in a supine position by an assistant while the dose was delivered using a 1 cm^3 syringe equipped with a 20 gauge animal feeding tube (Popper and Sons Inc., New Hyde Park, NY), which was inserted through the mouth gently until the tip was just above the proventricular opening of the gizzard. Following administration of the dose, each bird was individually housed in a glass metabolism cage with a 10 L volume (Kent Scientific, Litchfield, CT) designed for large rodents (>300 g). The cages were large enough to permit the birds to stand upright without restricting their movement. The metabolism cages were set up as a closed system such that all excreted radioactivity could be collected. Air lines were connected to the cages to permit delivery of oxygen (300 mL/min) and collection of expired air. A course wire mesh was used as the floor of the cage, which permitted the collection of fecal−urate (birds excrete both urine and feces through the cloaca, so the term fecal−urate will be used to refer to all waste products). The birds had free access to food and water for the duration of the test period.

Animal Dosing. For each treatment group, [^{14}C]CPTH was administered to three birds of each species as described in the sections above. At the conclusion of each exposure time, the birds were removed from their cages and euthanized via exposure to CO_2. Each carcass was removed from the euthanizing chamber, and a sample of whole blood was taken via cardiac puncture into a heparinized syringe. The carcass was then placed in a plastic bag and frozen (at −30 °C) until dissection could be performed. All procedures involving animals were carried out with the approval of the Animal Care and Use Committee. The food and water dishes were emptied into individual glass jars with Teflon lids and stored at 4 °C until analysis for ^{14}C content could be performed. Expired air (CO_2) and fecal−urate samples were placed in individual sample containers and stored until analysis. The cage was then washed with 2 L of deionized water, and the wash water was analyzed for ^{14}C content as per the procedures for drinking water below.

Collection of Expired CO_2. Expired CO_2 was collected by using two glass trapping vessels in series. Each contained 30 mL of a basic scintillation trapping solution (Carbon-14 Cocktail; R. J. Harvey Instrument Corp., Hillsdale, NJ). Expired air was initially collected from the birds in the 24-h exposure time period and found to contain no detectable amounts of ^{14}C. Therefore, the remainder of the treatment groups were housed with a wire mesh lid on the metabolism cage rather than a glass lid and no expired air was collected for those treatment groups. The behavior of the birds indicated that they were calmer when housed in the wire mesh topped cages.

Table 1. Sample Analysis Techniques Employed To Determine Total Radioactive Residue Levels in Various Matrices

matrix	direct LSC analysis	liquid sample	combustion analysis	homogenate
expired air	yes	no	no	N/A
drinking water	yes	yes	no	N/A
cage wash water	yes	yes	no	N/A
feed	no	no	yes	no
fecal–urate	no	no	yes	yes
brain	no	no	yes	yes
breast muscle	no	no	yes	yes
GI tract	no	no	yes	yes
heart	no	no	yes	yes
kidney	no	no	yes	yes
leg muscle	no	no	yes	yes
liver	no	no	yes	yes
lung	no	no	yes	yes
whole blood	no	no	yes	no

Tissue Collection. Each carcass was allowed to thaw slightly before proceeding with the necropsy procedure. An incision was made in the skin covering the abdomen, and the birds were skinned completely. A lateral incision was then made and a pair of scissors used to cut the breast bone on each side. The breast was removed and a portion of the muscle tissue removed from it with a scalpel. The heart, lungs, liver, gastrointestinal (GI) tract (from esophagus to cloaca with contents included), and kidneys were then individually removed. Next the brain was removed by cutting the skull laterally between the orbital sockets with a pair of scissors. A cut was then made from one eye to the other around the circumference of the anterior skull and the top of the skull removed. The brain was cut free from the spinal cord and removed. Last, a portion of the leg muscle tissue was removed using a scalpel. A pair of scissors was used to mince each tissue before it was placed into a separate preweighed glass homogenization tube. Fecal−urate samples were allowed to thaw and placed in a preweighed glass homogenization tube. Sample weights were recorded, and a measured volume of deionized water was added to each tube to produce approximately a 3:1 water-to-sample ratio. Each tissue was homogenized using a Teflon and glass homogenization tube and stored at −30 °C in individual glass vials until combustion analysis could be performed. Tissue and fecal−urate samples for individual birds were processed and stored separately and were not pooled. Feed samples were ground to a fine powder using a coffee grinder, weighed, and stored at −30 °C until analysis. Drinking water, cage wash water, and whole blood samples were directly analyzed as collected without further sample preparation steps.

Liquid Scintillation Counting Analysis. Depending on the nature of the sample matrix (liquid, solid, or homogenate), the samples were prepared for analysis and counted on the liquid scintillation counter (**Table 1**). Radioactivity was determined using a Packard Tri-Carb 1600TR liquid scintillation counter (LSC). Samples were counted in triplicate for 10 min (4−156 keV). Drinking water and cage wash water samples were analyzed for ^{14}C content by pipetting 1 mL into a 20-mL scintillation vial containing 20 mL of Scintiverse BD scintillation cocktail (Fisher Chemicals, Fair Lawn, NJ). The response of a background blank of deionized water was subtracted from each response and the total disintegrations per minute (DPM) determined for each sample.

Subsamples of each homogenate, feed, and whole blood sample were weighed in duplicate in porcelain combustion boats containing 0.5 g of mannitol (reagent grade; EM Science, Gibbstown, NJ). The samples were combusted in an R. J. Harvey model OX-600 biological oxidizer.

Oxygen and nitrogen flows were 350 mL/min. The combustion and catalyst zone temperatures were held at 900 and 680 °C, respectively, and samples were combusted for 4 min. The CO_2 produced was trapped in a ^{14}C cocktail (R. J. Harvey). The cocktail was transferred to a glass vial and counted on the LSC using the method described above.

Data Collection. For data obtained from direct measurement of aliquots of aqueous samples, the raw counts were corrected for counting efficiency by the LSC and reported as DPM. The DPM of a background sample of deionized water was subtracted from all results including control samples. The data were further corrected by subtracting the average DPM of control samples from those results obtained for test samples. This yielded a background-corrected value (BCV). The BCV was divided by sample mass or volume to give DPM per unit mass or volume for each sample.

Data obtained from combustion analyses were treated in a similar manner, with the following exceptions. The efficiency of the combustion apparatus was determined by combusting an aliquot of mannitol fortified with a known quantity of [^{14}C]CPTH at the beginning and end of each day's analyses. Recovered radioactivity was determined by counting on the LSC, and the percent recovery of DPM was calculated for each fortified sample. The average recovery of the two fortified samples was used as a correction factor for that day's analyses. All results obtained on that particular day were corrected for this efficiency value. A BCV was also obtained for each tissue type by subtracting the mean DPM value of the samples obtained from control animals from the observed DPM values for treated animals. Finally, a calculation was performed to relate the obtained result back to initial tissue weight instead of homogenate weight. This was accomplished through simple ratio calculation of unit mass of tissue per unit mass of homogenate.

Data Analysis. The data were analyzed on the basis of total radioactivity in each tissue type or "compartment". This value represents an estimate of the total DPM based on the average response for duplicate analyses of each subsample. This value incorporates any dilutions of the sample resulting from sample preparation. In most cases, the entire organ was processed as a tissue homogenate in water. Subsamples of breast muscle, leg muscle, and blood were analyzed and the resulting values used to estimate the total DPM for the entire tissue. In the case of blood samples, it was assumed that blood volume was ~10% of the body weight of the bird (*12*). For breast muscle and leg muscle, values of 26.9 and 8.9% of body weight were chosen for blackbird breast and leg; values of 22.7 and 10.5% were chosen for junco breast and leg, respectively (*13*). These values were used to calculate the percentage of administered dose found in each tissue compartment at each time point (**Table 4**).

The data were also evaluated on the basis of concentration of CPTH and metabolites in parts per billion of CPTH equivalents. CPTH equivalents are defined as unmetabolized CPTH and all metabolites of CPTH containing ^{14}C. During statistical analysis of the data, it was determined that a log transformation of the data produced a better linear regression result. This determination was based on inspection of the residuals and an evaluation of the R^2 values. Using nontransformed data, plots of the residuals demonstrated that the variability was not uniform, with the data points closer to time 0 having much larger residuals than those at later time points (data not shown). Residual plots of the log-transformed data produced much more uniform variability. In addition, using log-transformed data improved the R^2 values significantly. One purpose of performing this research was to develop tools to aid risk assessment of CPTH to sensitive and nonsensitive species. The data demonstrated a two-phase elimination profile, with a very rapid early elimination phase and a much slower secondary elimination phase. A nonlinear two-compartment model could have been used to describe the data very well. However, calculation of the half-life of elimination was a prime goal of the research. Therefore, a linear model using log of concentration as the response versus time was employed, and the data were evaluated from 0 to 4 h and from 4 to 24 h as two separate linear regressions.

The results of this transformation were also used to calculate elimination constants (K_{El}) and half-life of elimination ($t_{1/2}$) values for both elimination phases in each tissue or bodily fluid as well as combined values for whole body elimination. The slope of this line yielded the K_{El}. From this value, the half-life of elimination was easily found. Because a half-life is commonly defined as the amount of time for half of the remaining radioactivity to be excreted from the body, 100% elimination can never be mathematically achieved. A close approximation can be found using 7 times the value of $t_{1/2}$. This accounts for elimination of 99.2% of the radioactivity. The values for each statistic as well as the *p* value for the slope of the regression lines are given (**Tables 2** and **3**).

Table 2. Log DPM Linear Regression Results, K_{El}, and $t_{1/2}$ Values for Time 15 min to 4 h ($n = 12$ per Species)

tissue	R^2	slope	*p*	SE	K_{El}	$t_{1/2}$ (h)	95% CI of $t_{1/2}$	99.2% elimination
Dark-eyed Junco								
brain	0.840	–3.0129	0.0003	0.4598	6.939	0.10	0.08, 0.15	0.70
breast	0.830	–2.7576	0.0004	0.4360	6.351	0.11	0.08, 0.16	0.77
GI tract	*a*	–0.3237	0.4549	0.4092	*a*	*a*	*a*	*a*
heart	0.804	–2.1478	0.0007	0.3698	4.946	0.14	0.1, 0.22	0.98
kidney	*a*	–0.9618	0.1650	0.6204	*a*	*a*	*a*	*a*
leg	0.646	–2.4066	0.0055	0.6096	5.542	0.13	0.08, 0.27	0.91
liver	0.794	–1.9284	0.0008	0.3413	4.441	0.16	0.11, 0.25	1.12
lung	0.661	–1.4220	0.0047	0.3489	3.275	0.21	0.14, 0.44	1.47
blood	0.547	–2.3436	0.0137	0.7176	5.397	0.13	0.08, 0.36	0.91
carcass	0.501	–1.0753	0.0199	0.3581	2.476	0.28	0.16, 0.92	1.96
Red-winged Blackbird								
brain	0.949	–1.0049	<0.0001	0.0777	2.314	0.30	0.26, 0.36	2.10
breast	0.958	–0.8850	<0.0001	0.0614	2.038	0.34	0.3, 0.4	2.38
GI tract	0.387	–0.2169	0.0323	0.0839	0.500	1.39	0.77, 7.27	9.73
heart	0.929	–0.7642	<0.0001	0.0704	1.760	0.39	0.33,0.49	2.73
kidney	*a*	–0.1257	0.2182	0.0941	*a*	*a*	*a*	*a*
leg	0.958	–0.8233	<0.0001	0.0570	1.896	0.37	0.32, 0.43	2.59
liver	0.803	–0.3019	0.0003	0.0492	0.695	1.00	0.74, 1.51	7.00
lung	0.962	–0.5173	<0.0001	0.0344	1.191	0.58	0.51, 0.68	4.06
blood	0.927	–0.6039	<0.0001	0.0564	1.391	0.50	0.42, 0.62	3.50
carcass	0.945	–0.4467	<0.0001	0.0360	1.029	0.67	0.58, 0.81	4.69

[a] Slope not significantly different from zero; no values can be calculated.

Table 3. Log DPM Linear Regression Results, K_{El}, and $t_{1/2}$ Values for Time 4–24 h ($n = 12$ per Species)

tissue	R^2	slope	p	SE	K_{El}	$t_{1/2}$ (h)	95% CI of $t_{1/2}$	99.2% elimination
Dark-eyed Junco								
brain	*a*	–0.0466	0.2073	0.0336	*a*	*a*	*a*	*a*
breast	0.337	–0.0879	0.0592	0.0391	0.202	3.43	1.77, 49.12	24.01
GI tract	0.678	–0.1587	0.0039	0.0376	0.365	1.90	1.27, 3.76	13.30
heart	0.398	–0.0963	0.0406	0.0384	0.222	3.12	1.7, 18.98	21.84
kidney	0.414	–0.1310	0.0365	0.0508	0.302	2.29	1.27, 12.17	16.03
leg	0.327	–0.0681	0.0626	0.0308	0.157	4.41	2.27, 81.81	30.87
liver	0.610	–0.0770	0.0079	0.0209	0.177	3.92	2.49, 9.08	27.44
lung	0.614	–0.0508	0.0076	0.0137	0.117	5.92	3.78, 13.6	41.44
blood	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
carcass	0.698	–0.1425	0.0031	0.0323	0.328	2.11	1.43,4.01	14.77
Red-winged Blackbird								
brain	0.430	–0.0539	0.0122	0.0177	0.124	5.59	3.31, 17.8	39.13
breast	0.342	–0.0772	0.0269	0.0298	0.178	3.89	2.16, 20.27	27.23
GI tract	0.559	–0.0868	0.0031	0.0224	0.200	3.47	2.25, 7.55	24.29
heart	0.234	–0.0469	0.0635	0.0225	0.108	6.42	3.22, 139.59	44.94
kidney	*b*	–0.0049	0.8655	0.0280	*b*	*b*	*b*	*b*
leg	0.363	–0.0424	0.0225	0.0157	0.098	7.07	3.99, 31.75	49.49
liver	0.387	–0.0750	0.0182	0.0266	0.173	4.01	2.3, 15.59	28.07
lung	0.257	–0.0456	0.0533	0.0208	0.105	6.60	3.38, 147.76	46.20
blood	*b*	–0.0217	0.3724	0.0233	*b*	*b*	*b*	*b*
carcass	0.463	–0.0500	0.0089	0.0154	0.115	6.03	3.66, 17.02	42.21

[a] Values less than background; data not used. [b] Slope not significantly different from zero; no values can be calculated.

RESULTS AND DISCUSSION

Red-winged blackbirds and dark-eyed juncos were chosen to represent species that exhibit varied sensitivities to CPTH. Red-winged blackbirds, a target species, are highly sensitive to

Table 4. Mean Concentration (CPTH Equivalents) and Percent of Recovered Dose Values for Dark-eyed Juncos and Red-winged Blackbirds

		residue levels in juncos		residue levels in blackbirds	
tissue	time (h)	ppb CPTH (equiv)	% of dose	ppb CPTH (equiv)	% of dose
brain	0.25	7500	1.51	1900	1.94
	0.5	2600	0.56	1400	0.91
	1	730	0.17	1300	0.75
	4	39	<0.01	55	0.03
	8	7.2	<0.01	21	0.01
	12	9.1	<0.01	23	0.01
	24	11	<0.01	15	<0.01
breast	0.25	8000	15.05	2100	35.42
	0.5	3200	6.72	1900	22.58
	1	960	2.25	1700	18.13
	4	83	0.17	100	1.05
	8	7.8	0.02	32	0.34
	12	8.4	0.01	39	0.39
	24	7.3	0.01	26	0.22
GI tract	0.25	58000	46.99	6300	18.24
	0.5	53000	45.08	8600	19.93
	1	47000	39.54	11000	22.48
	4	8800	7.17	4500	7.98
	8	1200	0.84	2200	3.91
	12	2100	1.19	1500	2.05
	24	360	0.24	640	1.01
heart	0.25	11000	1.20	2000	1.48
	0.5	4600	0.53	1900	1.07
	1	2000	0.24	1600	0.74
	4	230	0.03	160	0.08
	8	64	<0.01	86	0.04
	12	79	<0.01	100	0.04
	24	48	<0.01	70	0.03
kidney	0.25	35000	3.03	6900	2.41
	0.5	20000	1.35	22000	5.40
	1	17000	1.31	16000	3.59
	4	13000	1.00	12000	2.59
	8	1400	0.09	11000	2.45
	12	5100	0.31	18000	4.13
	24	490	0.04	11000	2.20
leg	0.25	8500	7.34	1500	8.66
	0.5	3500	3.32	1500	6.05
	1	1600	1.67	1300	4.46
	4	95	0.10	98	0.33
	8	24	0.02	58	0.20
	12	22	0.02	49	0.16
	24	19	0.02	41	0.12
liver	0.25	19000	4.58	3600	4.08
	0.5	9600	2.35	4400	3.09
	1	4500	1.15	4900	3.19
	4	950	0.25	1700	1.10
	8	400	0.08	1900	1.05
	12	220	0.04	1300	0.68
	24	170	0.04	470	0.27
lung	0.25	12000	1.64	2300	2.42
	0.5	7400	1.03	2100	1.51
	1	3700	0.57	2100	1.19
	4	570	0.11	400	0.22
	8	230	0.03	410	0.23
	12	240	0.03	260	0.16
	24	160	0.03	270	0.14
blood	0.25	8600	7.32	1900	12.18
	0.5	3300	3.07	2100	9.19
	1	2200	2.22	2400	9.27
	4	*b*	*b*	300	1.18
	8	*b*	*b*	100	0.40
	12	*b*	*b*	190	0.66
	24	*b*	*b*	160	0.49

[a] Each number is the average of three observations. [b] Observed values were below values for control samples.

CPTH, having an LD_{50} of 1.8−3.2 mg/kg (*14*, *15*), whereas dark-eyed juncos, a nontarget species, are much less susceptible, with an LD_{50} of 162 mg/kg (*10*). Several variables were considered in the design of this experiment: CPTH dose level, ^{14}C activity in dose, route and method of exposure, and length of exposure time.

Selection of dose level, which was representative of a real-world exposure, was one parameter considered. The selection of an appropriate dose level was based upon ingestion of a single grain of 2% CPTH-treated rice bait. Previous research with CPTH indicated that a single treated rice grain would typically be sufficient to induce acute toxicity in sensitive species, and that by diluting treated rice 1:25 with untreated rice the desired exposure could be achieved (*2*). This is equivalent to a dose of ∼4 mg/kg for a 100-g bird. During study design, it was assumed that the weight of the more sensitive blackbirds would be ∼60 g. Therefore, a final dose of ∼250 μg of CPTH was selected. The dose was uniform for all birds and was not adjusted for differences in body weight. Another key consideration was the desire to produce no mortality during the course of the study. The dose level chosen was below the published LD_{50} values for juncos and below a level expected to produce mortality in blackbirds in <24 h. No mortalities occurred during the study as a result of CPTH toxicity: therefore, the dose seemed to be appropriate for the intended purpose.

The level of ^{14}C activity administered to each bird was based on the limit of detection (LOD) for the LSC and biological oxidizer. The limit of detection for the biological oxidizer was ∼50 DPM. Using this value, a method limit of detection for the samples was found to be ∼2000 DPM for a 10-g tissue sample. If the bird retained as little as 1% of the administered dose in its body and a small portion of that dose was contained in the tissue being analyzed, the response for that sample should be ∼10-fold above the method LOD.

The route and method of exposure were chosen to permit a reasonable assurance that the entire dose was ingested by each test animal. The predominant route of exposure in a field application is via ingestion; therefore, an oral gavage of CPTH in 100 μL of deionized water was used.

Sampling times were selected to provide a cross-section of exposure. Previously published data indicated that most of the CPTH would be excreted in <24 h (*8*, *16*). Information on rate of distribution would also be important; therefore, the sampling times were slanted toward shorter time periods rather than being evenly spaced over the 24-h test period. The times selected were 15 and 30 min and 1, 4, 8, 12, and 24 h postdose. The results for the 15- and 30-min and 1- and 4-h time points are found in **Figure 1**. No further time points are shown as >greater than 90% of the radioactivity had been excreted by the 4-h time point.

In most of the tissues and fluids collected from both bird species tested, a similar pattern was observed in plots of the radioactive residue (RR) as a function of time postdose. In the case of every tissue except blackbird kidney, >greater than 80−90% of the administered dose was excreted from the body of the test animal by 4 h after exposure. This supports the conclusion that CPTH is not retained in the carcass of exposed birds in significant quantities, with the exception of the RR in liver and kidney of exposed birds.

In the case of blackbird kidney (**Figure 2**), RR levels significantly higher than background levels were observed for the duration of the test. This level of radioactivity is approximately equivalent to a mean kidney concentration of 15 mg/kg of CPTH equivalents. A slight elevation of RR level was also observed in junco kidney (**Figure 2**) and in blackbird liver

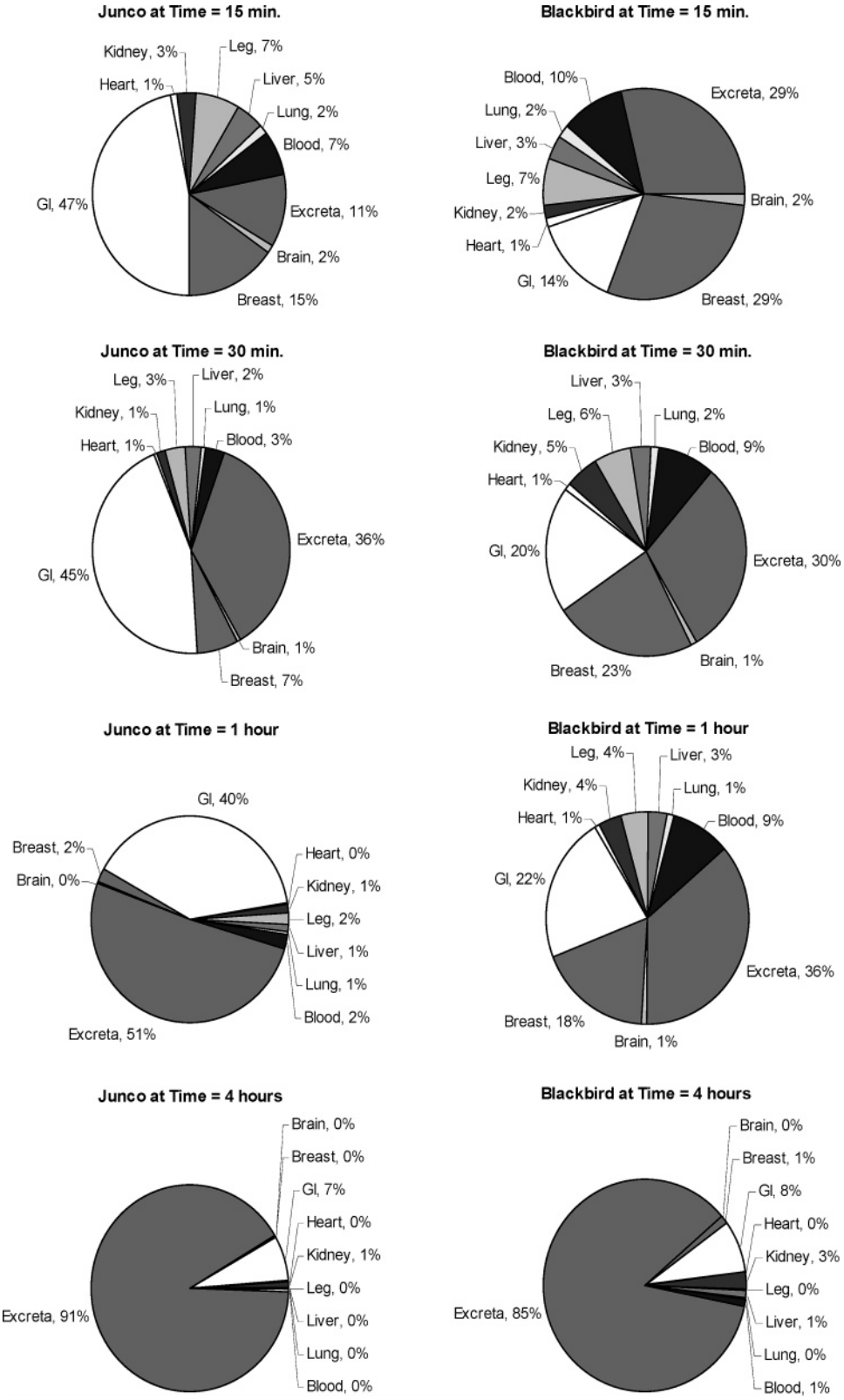

Figure 1. Percentage of radioactive CPTH and metabolites in each tissue at four sampling times.

(**Figure 3**). However, the results were not as pronounced as those for blackbird kidney. Possible reasons for this difference in elimination relate to potential binding of CPTH metabolites to these sensitive tissues. We are conducting in vitro studies in our laboratory to evaluate this possibility.

The plots of RR in the blood versus time also demonstrate one very distinct feature. There is a virtual lack of any type of uptake curve for CPTH (**Figure 4**). This suggests that CPTH delivered in this manner is readily absorbed and rapidly perfused through the body. This is further supported by a comparison of oral LD_{50} to intraperitoneal (ip) LD_{50} values for CPTH in starlings (*6*). The value for an oral exposure has been reported at 3.8 mg/kg, whereas that for an ip injection was found to be 3.5 mg/kg.

Examination of the half-life of elimination values (**Tables 2** and **3**) reveals that the radioactivity took longer to clear from

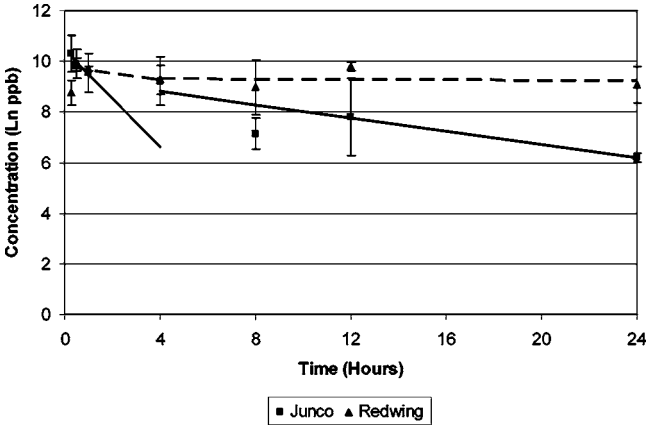

Figure 2. Logarithmic plot of elimination of radiolabeled CPTH and metabolites from kidney of red-winged blackbirds and dark-eyed juncos following a single oral dose ($n = 3$ per time point; error bars indicate standard deviation). Regression lines indicate the rate of elimination for junco (solid line) and blackbird (dashed line) for both the rapid and slow elimination phases.

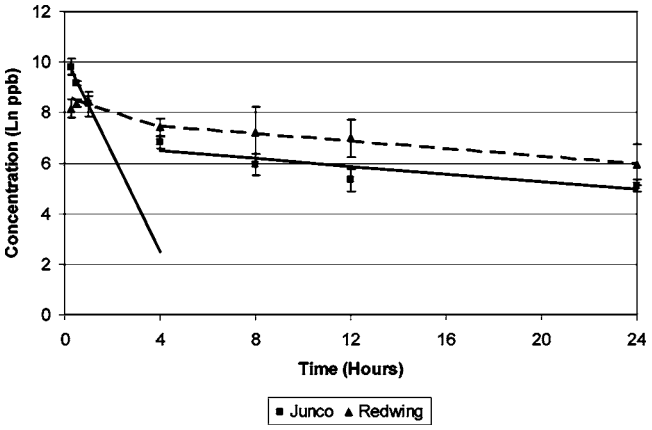

Figure 3. Logarithmic plot of elimination of radiolabeled CPTH and metabolites from liver of red-winged blackbirds and dark-eyed juncos following a single oral dose ($n = 3$ per time point; error bars indicate standard deviation). Regression lines indicate the rate of elimination for junco (solid line) and blackbird (dashed line) for both the rapid and slow elimination phases.

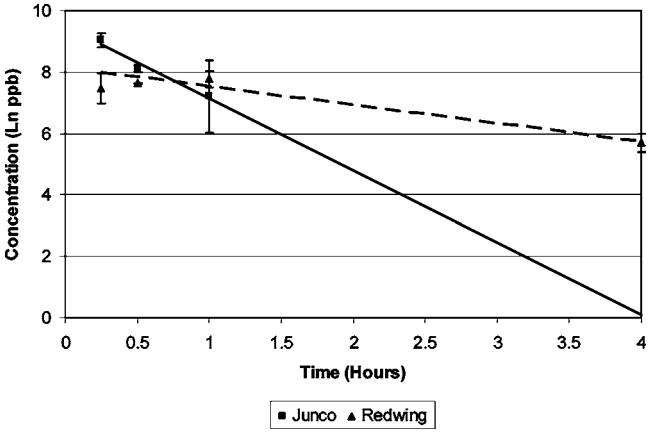

Figure 4. Logarithmic plot of elimination of radiolabeled CPTH and metabolites from whole blood of red-winged blackbirds and dark-eyed juncos following a single oral dose ($n = 3$ per time point; error bars indicate standard deviation). Regression lines indicate the rate of elimination for junco (solid line) and blackbird (dashed line) for the rapid elimination phase.

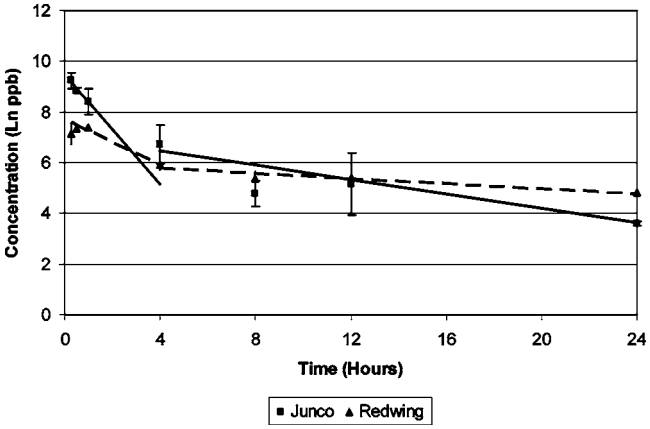

Figure 5. Logarithmic plot of elimination of radiolabeled CPTH and metabolites from whole body of red-winged blackbirds and dark-eyed juncos following a single oral dose ($n = 3$ per time point; error bars indicate standard deviation). Regression lines indicate the rate of elimination for junco (solid line) and blackbird (dashed line) for both the rapid and slow elimination phases.

blackbirds than from juncos as evidenced by the longer half-lives. This is further bolstered by the difference in whole body elimination rates between the two species (**Figure 5**). This could be partially due to the larger body mass of the blackbird as opposed to the junco. However, because the data were evaluated as concentrations and adjusted for body mass, it is equally likely that any differences observed are due to differential metabolism between the two species. The R^2 values for each tissue type in the first elimination phase demonstrated a reasonable fit to a linear model in most cases (**Table 2**). The R^2 values for the second elimination phase demonstrated a far less desirable fit for a linear regression (**Table 3**). As the residue levels approached background levels, the variability of the results increased, resulting in lower R^2 values overall. In the case of several tissue types, the slope of the regression line was found to not be significantly different from zero ($p = 0.10$). In the first elimination phase, junco GI tract and kidney and blackbird kidney did not meet the criteria for a linear regression. Therefore, no elimination rate constants or half-lives were calculated for these tissues. In the second elimination phase, rate constants could not be generated for junco brain and blood or for blackbird kidney.

Elimination of CPTH from the kidney of both bird species was slower than for most other tissue types. The difference in elimination was even more pronounced for blackbird than for junco (**Figure 2**). This further supports the hypothesis that CPTH is metabolized differently in junco than it is in blackbird. The slope of the line for junco kidney very closely mirrors that for liver tissue (**Figures 2** and **3**). Results of a two-tailed *t* test reveal that the slopes of the elimination curves, which are directly related to the half-life, for junco kidney and junco liver are not statistically different ($p = 0.3239$). Conversely, the half-life of elimination for blackbird kidney could not be calculated because it has no significant slope.

Previous research has hypothesized that the mode of action for CPTH toxicity involves damage to the kidneys (*16*), more specifically, damage to proximal tubular cells of the kidney (*6*, *7*, *11*). Additionally, observations of increased blood uric acid levels (*7*) have been made. The appearance of uric acid deposits in the abdominal cavities of exposed birds is also used as a method of determining exposure in certain cases (*6*, *17*, *18*).

Renal damage of this type can be indicative of a highly reactive chemical that may be able to covalently bind to tissues. Such a highly reactive chemical species could cause the extensive tissue damage observed in the previously mentioned studies, resulting in a failure of normal kidney functions and the subsequent uric acid increases. The fact that the RR in the kidney of the more sensitive blackbird species did not change significantly over time in comparison to the that of the less sensitive junco points to the kidney as a possible site of action. This observation is consistent with the hypothesis that covalent binding is occurring in the kidney of blackbirds to a greater degree than in the kidney of exposed juncos, especially when compared to the rapidly declining RR in the other compartments of the blackbird.

Although it is apparent that CPTH or more likely one of its metabolites is strongly retained by the kidney of red-winged blackbirds, the bulk of the parent and metabolites are rapidly excreted from the bodies of both sensitive and nonsensitive species. Factoring in observations from previous studies that time to death is typically >24 h (*6*), it seems fairly clear that carcasses of exposed birds found in the field are unlikely to contain significant residues of CPTH or its metabolites. These findings are significant with respect to estimating potential secondary exposure of wildlife that may consume CPTH-containing pest bird carcasses. The results presented further suggest that future research aimed at elucidating the bioactivation pathway by which CPTH is retained by renal tissues of susceptible bird species would greatly increase our understanding of the mode of action of CPTH and possibly lead to the development of more effective and safer avicides.

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