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Dose–Tissue Relationships for Dieldrin in Nestling Black-Billed Magpies

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Environmental toxicologists have urged researchers to explicitly identify relationships between dose level and tissue residues for wild birds (Peakall 1996). If relationships between dose and tissue residues are understood, then comparisons of differences in temporal and spatial dieldrin levels are more useful (Blus 1995). We believe it is important to understand the relationship between dose (exposure) and tissue residues for monitoring contaminants in a field environment, particularly when linking exposure to ecological effects is a goal.

Using non-lethal tissue samples (Fossi et al. 1994) to monitor wildlife exposure offers several benefits compared to sampling tissues that require sacrificing the study animal. Non-lethal samples allow researchers to sample the same individual over time and to examine ecological effects of exposure after the sample is collected (Frank and Lutz, 1999). Blood plasma is commonly used as a non-lethal sample, and has been correlated to residue burdens in humans (Radomski et al. 1971) and several vertebrate species (Fossi et al. 1994).

We conducted an "in-nest" dosing study using dieldrin and nestling black-billed magpies (*Pica pica*) to 1) examine the relationship between dose and tissue residues, and 2) evaluate lethal and non-lethal tissue samples as measures of exposure. Our study was conducted during the magpie breeding season in 1997 as part of a larger project evaluating magpies as biomonitors for organochlorine contaminants at the Rocky Mountain Arsenal National Wildlife Refuge (Refuge) in Denver, Colorado. The information gained from this study will be used to aid in the interpretation of magpie tissue samples collected on the Refuge and in the design of a long-term biomonitoring program.

MATERIALS AND METHODS

The Refuge is a 6,900 ha U. S. Army facility formerly used for the production of weapons and pesticides, which is currently managed by the U. S. Fish and Wildlife Service as a wildlife refuge. Certain areas near production facilities and

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storage basins in the interior of the Refuge are contaminated with organochlorines (aldrin, dieldrin, DDT, DDE, endrin, gamma- and alpha-chlordane) and heavy metals (arsenic and mercury). Dieldrin is the primary contaminant of concern for wildlife because of its toxicity, persistence, and widespread distribution on the Refuge. We conducted the dosing study using nests in the relatively uncontaminated periphery of the Refuge. Dieldrin concentrations in magpie eggs from this area are not different than those from an offsite control area (Cherry Creek State Park) in the Denver area (B. Vander Lee, unpublished data). Habitat on the Refuge consists primarily of grasslands interspersed with cottonwood (*Populus sargenttii*) woodlots and locust (*Robinia neomexicana*) thickets.

Our dosing solutions were prepared by dissolving crystalline dieldrin (98-99% pure) into corn oil and administered 5 dosing levels: control (corn oil only), 0.01, 0.10, 1.00, and 10.00 mg/kg body weight. We selected nests for dosing randomly from active nests in uncontaminated areas of the Refuge and assigned dose levels randomly to each nest. Dosing solutions were administered orally using a gavage needle at a rate of 0.40 ml / 100 g body weight. All nestlings in each nest received the same dose level.

We individually marked nestlings with plastic leg bands. Since magpie eggs hatch asynchronously, we measured the age of the nest as the number of days since the first egg hatched. Nestlings were weighed, measured tarsal length, and dosed five times, on days 5, 9, 13, 17, and 21. We collected dosed nestlings on day 23. We euthanized nestlings in a CO_2 chamber and collected blood immediately after death using a heparinized syringe and heart puncture. Blood was centrifuged for 15 minutes, separated, and plasma frozen at -10° C. We collected brain, liver, and abdominal fat samples and froze them for storage.

Plasma samples were analyzed for dieldrin by Denver Wildlife Research Center, Denver, Colorado, using gas chromatography with electron capture detection. The method-reporting limit (MRL) was 112 ppb for a 1 ml sample. E. A. Engineering Science and Technology in Sparks, Maryland analyzed brain, liver, and fat samples for dieldrin. The MRL was 2 ppb for a 2 g sample. All laboratory work met Quality Assurance and Quality Control protocols for the Refuge established by the U.S. Army, U.S. Fish and Wildlife Service, and the Environmental Protection Agency. We present values on a wet weight basis unless otherwise noted. For samples with residue concentrations below the MRL, we used a value of 50% of the MRL in statistical analysis.

When sample distributions were skewed, we transformed data ($Log_{10}(x+1)$) prior to statistical testing. To insure that nestlings were in similar condition at the start of the dosing study, we calculated a condition index (weight/tarsal length) for all nestlings using measurements from day 5 and compared condition indices among dose levels with one-way analysis of variance (ANOVA). We used one-way analysis of variance to test for differences in tissue residues and weight gain (day

5-21) among dose levels. When overall F-tests were significant, we used Tukey's multiple comparison method to separate means. We used least squares linear regression to investigate relationships between the amount of dieldrin (mg) given to each magpie nestling and residues in tissues. For each tissue, we calculated both simple and quadratic models and used an F-test to determine if the quadratic term contributed significantly to the model. We used similar procedures to investigate relationships between plasma residues and residue in the other tissues. We used S-plus (Mathsoft 1997) for all statistical tests and considered probability values <0.05 significant. While statistical results for tissue residues are based on transformed values, we present non-transformed data in tables and text as means (standard errors).

RESULTS AND DISCUSSION

We dosed and collected 38 magpie chicks; sample sizes ranged from 5 to 10 (Table 1). Condition indices at day 5 did not differ among dose levels (Table 1), suggesting there were no differences in the initial condition of nestling magpies. While the overall F-test for weight gain was significant, we found no clear pattern of weight gain by dose level (Table 1). In general, weight increased between day 5 (48.8(7.9) g) and day 17 (151.9(24.6) g), and stabilized between day 17 and day 21 (161.3(26.2) g). Previous researchers (Jefferies and Davis 1968, Porter and Wiemeyer 1972, Henny and Meeker 1981) have noted weight loss in birds dosed with organochlorines, and suggested it was due to disturbance of fat metabolism (Jefferies and Davis 1968). We did not observe any differences in weight gain among dose levels, perhaps because the time scale of our work (23 days) was relatively short.

Tissue residues varied among dose levels (Table 2). Fat and plasma tissues provided the greatest ability to detect differences among the dose levels, as we were able to differentiate among the three highest dose levels (> 0.10 mg/kg body wt.). In brain and liver tissues, we were only able to differentiate the highest dose (brain) and 2 highest doses (liver, Table 1). We were unable to differentiate differences between control and the lowest dose (0.01 mg/kg body wt.) for any tissue, suggesting that the lowest dose is similar to background levels of dieldrin for magpies in uncontaminated areas on the Refuge.

For all tissues, dieldrin concentrations were positively related to the quantity of dieldrin that was administered to nestlings (Fig. 1). Addition of the quadratic term significantly improved model fit for all tissues. Fat and liver showed convex curves (Fig 1.) while brain and plasma residues showed concave curves. Because of its lipophilic nature, dieldrin accumulated in fat and liver tissues even at low doses. At high doses further accumulation appeared limited and notable amounts of dieldrin became evident in the plasma and brain. It appears that dieldrin in nestling magpies is compartmentalized into lipid-rich tissues until a threshold is reached, and then becomes available to other body tissues.

Table 1. Mean condition index (weight (g) / tarsal length (mm)) on day 5, weight gain (day 5-21), and mean quantity dieldrin (mg) administered to 38 nestling black-billed magpies in 5 dose levels at the Rocky Mountain Arsenal National Wildlife Refuge, Colorado from May-June, 1997.

Dose (mg/kg body wt)	N	Condition Index (g/mm)	Weight Gain (g)	Mean Dieldrin (mg)
Control	7	2.08 (0.11)	116 (6) AB[a]	0.00 (0.00)
0.01	7	2.14 (0.07)	133 (6) A	0.01 (0.00)
0.10	5	2.08 (0.11)	95 (10) B	0.06 (0.01)
1.00	10	2.05 (0.08)	104 (4) B	0.58 (0.02)
10.00	9	2.03 (0.07)	113 (5) AB	5.94 (0.19)
P		0.919	< 0.001	

[a]Dose levels with the same letter are not significantly different ($P < 0.05$) based on Tukey's procedure.

Even at low dose levels, nestling magpies accumulated high residue levels in fat (Fig. 2), which may place them at increased risk during periods of fat mobilization (such as fledging) as organochlorines are shifted to other tissues, and the brain in particular (Jefferies and Davis 1968). Thus, we suggest nestling magpies at even low doses are at risk from dieldrin and stress the importance of knowing the nature of dose-tissue relationships.

Dieldrin concentrations in plasma were significantly related to concentrations in each of the other tissues (Fig. 2). We also found the best fitting relationship with dose using plasma (Fig 1.), and suggest that plasma is a useful non-lethal tissue for monitoring organochlorine contaminants in wildlife (Friend et al. 1979, Capen and Leiker 1979). Previous work suggests that organochlorines in plasma might

Table 2. Dieldrin concentrations (ug/g wet weight) in brain, liver, fat, and plasma of 38 nestling black-billed magpies dosed with 5 levels of dieldrin at the Rocky Mountain Arsenal National Wildlife Refuge, Colorado from May-June, 1997. Statistical results are based on transformed data ($Log_{10}(x+1)$).

Dose (mg/kg body wt)	N	Brain (ug/g)	Liver (ug/g)	Fat (ug/g)	Plasma (ug/g)
Control	7	0.02 (0.00) A[a]	0.09 (0.01) A	2.33 (0.46) A	0.01 (0.01) A
0.01	7	0.02 (0.00) A	0.04 (0.01) A	0.76 (0.17) AB	0.00 (0.00) A
0.10	5	0.03 (0.01) A	0.11 (0.02) A	3.49 (0.99) B	0.02 (0.01) B
1.00	10	0.19 (0.03) A	0.92 (0.06) B	34.65 (2.36) C	0.24 (0.02) C
10.00	9	2.82 (0.38) B	6.33 (0.71) C	419.88 (43.10) D	2.56 (0.19) D
P		<0.001	<0.001	<0.001	<0.001

[a]Dose levels with the same letter are not significantly different ($P < 0.05$) based on Tukey's procedure.

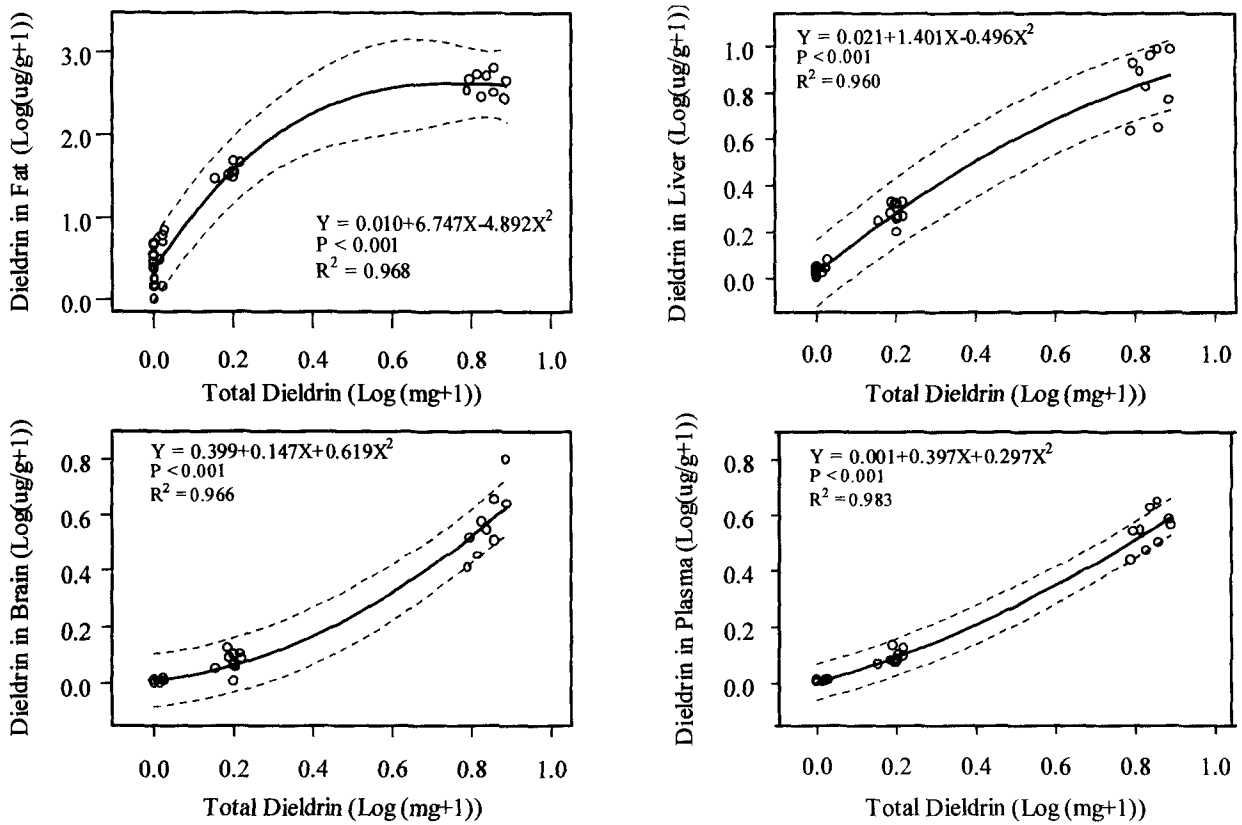

Figure 1. Relationships between total dieldrin (mg) administered to 38 nestling magpies and resulting tissue residues (ug/g wet weight). Values were transformed ($Log_{10}(x+1)$) for analysis. Dashed lines represent 95% confidence intervals.

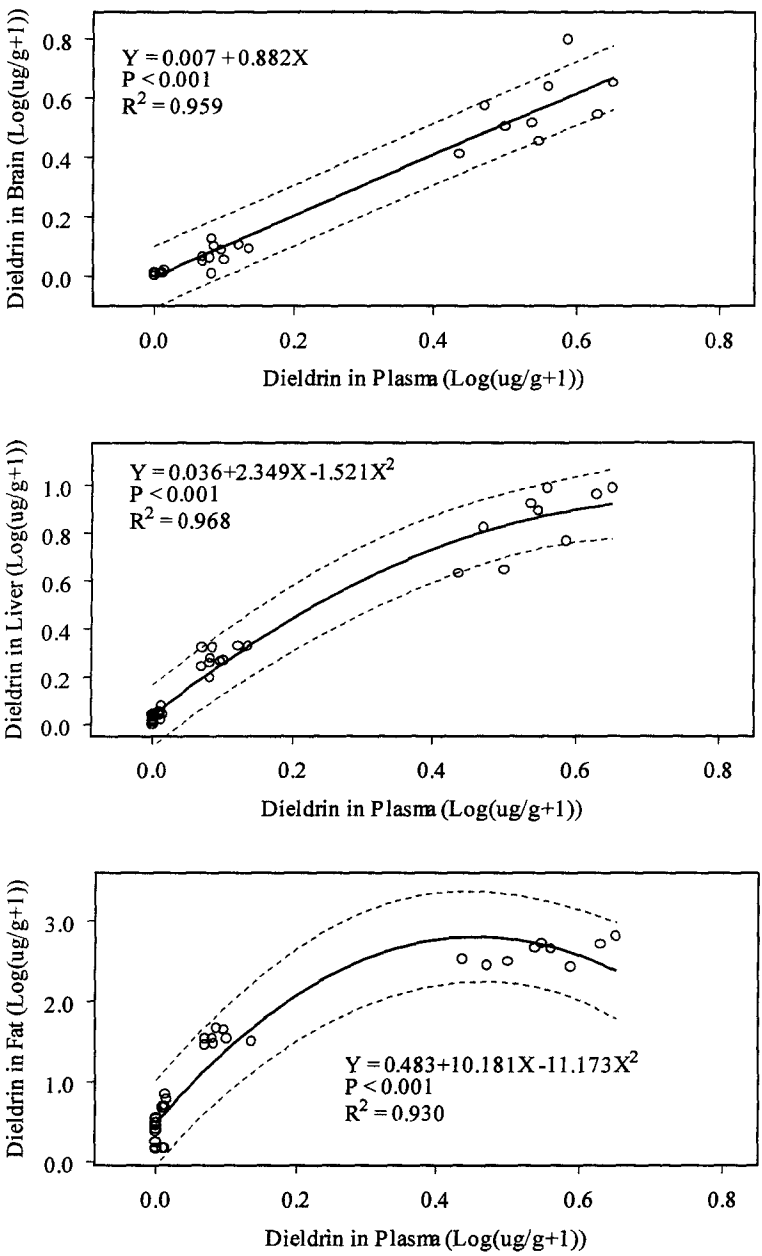

Figure 2. Relationship between dieldrin concentration (ug/g) in plasma and brain, liver and fat tissues from 38 nestling magpies administered 5 dose levels of dieldrin. Values were transformed ($\log_{10}X+1$) for analysis. Dashed lines represent 95% confidence intervals.

be variable through time and sensitive to changes in lipid content in the body (Capen and Leiker 1979). It is important to note that in our study, magpies were sampled when their weight was stable, and they were probably not accumulating or mobilizing fat.

We suggest that monitoring dieldrin in nestlings prior to fledging may reduce seasonal variability due to fat cycling and serve as a reliable, non-lethal technique to assess exposure to dieldrin. Given the relationship between plasma and brain levels of dieldrin, if dieldrin brain residues of 4-5 ug/g are used as diagnostic of lethality (Blus 1995) our data suggest that 2.51 ug/g (geometric mean, Fig. 2) in plasma could be indicative of potentially lethal exposure in 23 day old magpie chicks. Our data (B. Vander Lee, unpublished data) on home range sizes indicate that magpie pairs forage over 70-170 ha so it seems reasonable to use plasma values in chicks as representative of this area.

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