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TOXICITY OF COMPOUND 1080 TO MAGPIES AND THE RELATIONSHIP OF DOSE RATES TO RESIDUES RECOVERED

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ABSTRACT: The acute oral LD_{50} of Compound 1080 to magpies was estimated at 1.78 mg/kg indoors, 1.91 mg/kg outdoors in summer, and 2.30 mg/kg outdoors in winter. *Postmortem* 1080 residues were detected in 75 of 76 treated birds. Higher doses yielded higher 1080 residues. Within dose levels, birds surviving longer carried lower residues. In a separate test, an average residue of 0.09 ppm was found in 8 birds treated at 1.59 mg/kg and euthanized 24 h post dosing. The adjusted dietary LC_{50} of Compound 1080 to magpies tested indoors was estimated at 16 ppm. During LC_{50} tests, the influence of 1080 on food consumption and bird weight varied. Birds receiving low doses were unaffected and those receiving high doses died quickly. Birds that were affected but did not die quickly, usually lost weight but only slightly reduced food intake. All birds that died had detectable 1080 residue in breast muscle. Birds fed higher 1080 dietary concentrations probably exhibited higher residues *postmortem*. Our adjusted average LD_{50} (2.12 mg/kg) appeared somewhat higher than reported in the literature; nonetheless, magpies are very sensitive to 1080. No sex differences were noted. Age, metabolic influences, or cold temperatures, might explain the high LD_{50} value estimated for winter. The detection of 1080 residue in tissue samples is a useful tool for assessing 1080 exposure in magpies—but it might not be unequivocal.

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INTRODUCTION

For several years we have studied the use of Compound 1080 (sodium fluoroacetate) as a predacide in livestock protection collars (LPCs). These investigations resulted in registration (Reg. No. 56228-22) of the Compound 1080 Livestock Protection Collar by the U. S. Environmental Protection Agency (EPA) to control coyote predation (Moore 1985). LPCs cover the throats of sheep and goats. When coyotes attack the collared animals, they usually bite and puncture the collars and are killed (Connolly and Burns 1990).

Our studies included assessment of nontarget hazards to scavengers that might feed on coyotes (*Canis latrans*) killed by LPCs or on contaminated carcasses of collared livestock. Magpies (*Pica pica*) were chosen for testing because they are a common, widely distributed scavenger in the western United States and are more sensitive to 1080 than most other scavenging birds. Ward and Spencer (1947) reported LD_o and LD_{100} values for 1080 to magpies of 0.6 and 1.3 mg/kg, respectively. Atzert (1971) reported LD_{50} values for 7 other species of scavenging birds ranging from 1.25-5.00 mg/kg for golden eagles (*Aquila chrysaetos*) to about 15.00-20.00 mg/kg for black vultures (*Coragyps atratus*) and turkey vultures (*Cathartes aura*). More recently, Hudson et al. (1984) reported an LD_{50} of 3.54 mg/kg [95% confidence interval (ci) = 0.498-25.10] for golden eagles.

Since wild magpies live under various environmental conditions and other researchers have reported that the toxicity of 1080 to some species varies with temperature (Chenoweth 1950, McIlroy 1981, Oliver and King 1983, Eastland and Beasom 1986), we conducted LD_{50} tests under different conditions to determine if such variations affected the sensitivity of magpies to 1080. Additionally, we estimated the dietary LC_{50} for 1080 to magpies caged indoors, and calculated relationships between dose rates and tissue residues found in test birds in both LD_{50} and LC_{50} studies.

The studies were conducted to better interpret results from our nontarget hazard assessments and to provide more information about the toxicity of 1080 to magpies. The results were reported to EPA in documents supporting LPC registration.

MATERIALS AND METHODS

General

Studies were conducted between August 1981 and September 1984 at a research facility of the Denver Wildlife Research Center (DWRC) near Logan, Utah. Magpies were live-trapped on the area, and were randomly assigned by sex to test and control groups. Sex was predicted by bird weight prior to testing and was confirmed by *postmortem* examination. Commercial mink feed (MF) from Fur Breeders Agri Co-op. Assoc., Logan, UT[1] was used for the maintenance and test diets. The Compound 1080 (Tull Chemical Co., Oxford, AL) used to prepare test solutions had a nominal concentration of at least 90% active ingredient (ai). The nominal concentration was used to calculate test formulations. Compound 1080 was weighed on an analytical balance, and mixed with distilled water to make stock solutions at the highest desired concentration. Stock solutions were successively diluted to prepare dose solutions and test diets. LD_{50} trials were conducted under three experimental conditions—indoors, outdoors in summer, and outdoors in winter.

Observed signs of 1080 poisoning were recorded, and tissue samples of breast muscle and gizzard-stomach (combined) were collected and frozen for 1080 residue analysis immediately after test birds died. Samples for 1080 residue analyses were also collected from some birds that survived tests. Also, in a test separate from the LD_{50} determinations, all birds that survived 24 h at a mid-range oral dose level (1.59 mg/kg) were euthanized and sampled to determine residue levels in survivors. Data from the survivors of this test were not used in LD_{50} calculations. LD_{50} calculations were performed by DWRC statisticians following methods of Thompson and Weil (1952).

Test diets from LC_{50} studies and tissue samples from magpies were analyzed for 1080 content at the DWRC following procedures of Okuno et al. (1982). Test diets were analyzed with the following modifications: for each diet

[1] Mention of commercial products does not imply endorsement by the United States Government.

analysis, 2 g of material were placed in a 25 x 150-mm screw-cap culture tube; 40 ml of extraction solvent were added and the capped tube placed in an ultrasonic bath for 30 minutes; the tube was centrifuged and a 2-ml aliquot was removed amd placed in a 15 x 110-mm screw-cap tube with 1.6 ml of water; the procedure then continued as usual.

Indoor LD_{50} Tests

Initially, a range-finding test was conducted to determine dose levels for testing. Magpies were captured and held overnight in individual cages (48 x 20 x 30 cm) with water, but not food. The following day, 4 birds were orally gavaged at each of 3 dose levels (0.5, 1.0, and 2.0 mg/kg). Data from the 4 birds dosed at 1.0 and 2.0 mg/kg were subsequently used in LD_{50} calculations for birds held indoors. For the remaining indoor tests, magpies were held in individual cages (60 x 28 x 43 cm) under continuous light at about 16-20°C. Magpies were caged for a 7-day acclimation period, a 7-day test period, and a 7-day posttest observation period. The birds received food and water *ad libitum* (changed daily), but were fasted 24 h before dosing. Based on the range-finding test, additional birds were orally gavaged at 7 dose levels [0.00 (control), 1.00, 1.26, 1.59, 2.00, 2.52, and 3.17 mg/kg]. Control birds were gavaged with deionized water. Ten birds (including those from the range-finding test) were used at each dose rate. Dates, number of birds used, and dose rates (mg/kg) were: on 15 July 1981, 4 at 1.00 and 4 at 2.00 (range finding); on 15 to 28 Sept 1981, 5 at 0.00, 3 at 1.00, 5 at 1.26, 5 at 1.59, and 3 at 2.00; on 3 to 16 Nov 1981, 5 at 0.00, 3 at 1.00, 5 at 1.26, 5 at 1.59, and 3 at 2.00, on 24 Feb to 5 March 1982, 10 at 2.52; and on 25 to 31 May 1982, 10 at 0.00, and 10 at 3.17.

Outdoor LD_{50} Tests

Magpies were held in groups of 5 in sheltered outdoor pens (3.7 x 1.2 x 1.8 m) that contained perches. The feeding and watering schedule, dose rates, and birds per dose rate were similar to those used in the indoor tests. Two tests were conducted during each season with 5 birds used at each dose rate per season. Tests were conducted in Aug-Sept 1982 (summer) and Dec 1982-Jan 1983 (winter). Temperatures on dosing dates ranged from 6 to 33°C in summer and from -18 to 4°C in winter.

Indoor LC_{50} Tests

Two LC_{50} tests were conducted under captive conditions similar to those used for indoor LD_{50} tests. Six birds each were fed MF treated with 1080 at concentrations of 0.0, 2.5, 5.0, 10.0,and 20.0 ppm in Test I (1-13 Sept 1984) and 0.0, 40.0, and 80.0 ppm in Test II (17-29 Sept 1984). Control birds were fed MF containing no 1080. The second test was conducted because only 1 bird died in test I.

To prepare diets, measured amounts of stock solution were diluted appropriately and mixed with the proper amount of MF. Three mg/ml of rhodamine B dye in deionized water were added to each dilution to mimic the LPC formulation being tested. Prepared test diets were frozen in packages sufficient for a daily feeding; individual packages were later thawed as needed. Food consumption per bird was determined by weighing food into and out of each cage daily. Tests consisted of a 7-day acclimation period, a 5-day treatment period, and a 3-day post-test observation period. Birds were weighed at the end of each period and survivors were euthanized at the end of the study. Breast muscle was collected for 1080 residue determinations as soon as possible after a bird died. LC_{50} calculations were made by DWRC statisticians using binomial methods of Stephen (1977).

RESULTS

Purity and Diet Concentrations

The technical Compound 1080 used in the study was found to contain an average of 94.5% ai (n = 4, range = 89-100%); 5% higher than the nominal concentration of 90%. The 1080 concentrations found in test diets averaged about 80% of expected (n = 16, range varied with dose level).

LD_{50} Tests

The progression in signs of intoxication in 1080-treated birds included apparent nervousness, lethargy, ataxia, recumbency, seizures, and death. Lethargic birds sometimes remained motionless with eyes closed and feathers fluffed for up to several hours.

Based on the 95% confidence intervals, our estimated LD_{50}s for 1080 to magpies (Table 1) showed little difference between the values for indoors and outdoors in summer (1.78 and 1.91 mg/kg), whereas the value for outdoors in winter (2.30 mg/kg) appeared higher. These values yielded an average of 2.00 mg/kg. If adjusted for 94.5% ai in Compound 1080, an average LD_{50} of 2.12 mg/kg is indicated.

Analysis of tissues from the 76 birds that died during LD_{50} tests and from 2 that died during the 24 h test showed a wide range in residues recovered [not detected (nd)-3.00 ppm]. The lower detection limit for the analytical procedure was 0.05 ppm. Only one bird that died after receiving a 1080 dose showed no detectable residue in breast or gizzard-stomach tissues. This bird remained alive for an unusually long period (3 days) after treatment and probably metabolized and

Table 1. Estimated LD_{50} of 1080 to magpies under 3 experimental conditions, 1981-1983.

Doses[a] (mg/kg)	LD_{50} test results (deaths/birds tested)		
	Indoors	Outdoors	
		Summer	Winter
3.17	10/10	10/10	10/10
2.52	9/10	7/10	6/10
2.00	8/10	5/10	2/10
1.59	3/10	3/10	1/10
1.26	0/10	2/10	0/10
1.00	0/10	0/10	—[b]
0.00	0/30	0/13	0/10
LD_{50} (mg/kg)	1.78	1.91	2.30
95% ci	1.61-1.98	1.66-2.20	2.06-2.56

[a]Based on 90% ai for Compound 1080. Subsequent analysis indicated 94.5% ai.
[b]No magpies tested at this dose level.

excreted the residual 1080. All other birds surviving treatment appeared normal after about 48 h. None of 43 control birds died, and of 9 that were sampled for residue, none contained detectable amounts of 1080.

Eight of 10 magpies survived a 1080 dose of 1.59 mg/kg for 24 h and were then euthanized. They showed an average residue of 0.09 ppm (range = nd-0.27 ppm) in both tissues analyzed. One of these birds exhibited signs of intoxication, and probably would have died. None of the remaining 7 showed signs of intoxication; they probably would have survived.

At the 1.59 to 3.17 mg/kg dose levels there was a positive relationship between dose and *postmortem* residue in magpies, i.e., higher doses yielded higher residues. Linear regression equations for the relationships were:

Gizzard-stomach residue = -0.530 + 0.563 (dose rate)
Muscle residue = -0.278 + 0.397 (dose rate)

Correlation coefficients for both regressions ($r = 0.60$) were significant ($P > 0.01$). Residues varied within dose rates; some of the variation was associated with the length of time between dosing and death. Dose rates of 2.52 and 3.17 mg/kg showed significantly higher average residues for birds that died on treatment day versus birds that died subsequently (Table 2).

LC_{50} Tests

The treated-diet tests showed an estimated LC_{50} for 1080 to magpies of 20 ppm (96.9% ci = 10-40 ppm; Table 3); adjustment to 80%, as indicated by analysis, yields a value of 16 ppm. Affected magpies exhibited signs of intoxication similar to those described earlier.

Most birds that consumed test diets lost weight. Weight loss became noticeable at the 5.0 ppm dose rate and became more pronounced at higher doses. Most birds that survived gained weight when returned to a diet without 1080 (Table 4). There appeared to be no consistent influence of 1080 on food consumption at dose levels up to 20 ppm; some birds increased, and others decreased food consumption. At the 40 and 80 ppm doses, birds stopped eating, lost weight, and died quickly. post-treatment observations could not be made. One bird fed at 20 ppm 1080 was obviously ill for 2 days before death. It reduced food consumption by over 30 g per day and lost 41 g.

Total amounts of 1080 consumed by birds increased through the 20 ppm dose level. Birds treated at 40 and 80 ppm died quickly and actually consumed less 1080 than the birds dosed at 20 ppm (Table 4).

Residues of 1080 were found in breast muscle from the 14 birds that died during the LC_{50} tests; none was detected among the 12 control birds. Average residue levels appeared to increase with treatment level. The linear regression equation for the relationship was:

Breast muscle residue = -0.239 + 0.014 (dose rate).

The correlation coefficient for the regression ($r = 0.50$) was not significant at the 90% level.

DISCUSSION

LD_{50} Tests

Our estimated LD_{50} for Compound 1080 in magpies under 3 experimental conditions, an adjusted average of 2.12 mg/kg, was substantially higher than the LD_{100} (1.3 mg/kg) reported by Ward and Spencer (1947). We obtained a higher

Table 2. Average *postmortem* 1080 residue for magpies dosed with Compound 1080 during LD_{50} studies for birds dying on dose day[a] or later.

1080 dose[b] (mg/kg)	Day of death post dosing	Average 1080 muscle residue found (ppm): Breast muscle(SD)[c]	Gizzard-stomach(SD)
3.17	Dose day (n=21)	1.16 (.32)	1.52 (.56)
	Next day (n=9)	0.64 (.26)	0.90 (.21)
	Significant	(P > .05)	(P > .05)
2.52	Dose day (n=12)	0.84 (.21)	0.82 (.34)
	Next day (n=10)	0.50 (.25)	0.69 (.26)
	Significant	(P > .05)	(P > .05)
2.00	Dose day (n=3)	0.70 (.35)	0.86 (.47)
	Next day (n=12)	0.39 (.15)	0.40 (.18)
	Not significant	(P = 0.26)	(P = 0.24)
1.59	Dose day (n=1)[d]	0.73	0.70
	Next day (n=6)	0.23	0.26
	Not tested		

[a]Birds that died within 24 h of dosing.
[b]Based on 90% ai for Compound 1080. Subsequent analysis indicated 94.5% ai.
[c]SD = Standard Deviation.
[d]Insufficient number for a statistical test.

Table 3. Concentration of 1080 in diet, number of birds, day of death, and sex of birds tested in magpie LC_{50} determination.

1080 concentration in diet (ppm)[a]	Birds tested and sex (M or F)	Day of death and sex of bird (M or F) Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Total deaths
0	12 (5M, 7F)							0
2.5	6 (4M, 2F)							0
5	6 (4M, 2F)							0
10	6 (0M, 6F)							0
20	6 (5M, 1F)		1 (M)				1 (M)	2
40	6 (1M, 5F)	5 (1M, 4F)	1 (F)					6
80	6 (4M, 2F)	5 (3M, 2F)		1 (M)				6

LC_{50} = 20 ppm (96.9% confidence interval of 10-40 ppm)

[a]Based on 90% ai for Compound 1080. Subsequent analysis indicated 94.5% ai.

value in winter. This decreased sensitivity might have been related to bird age, other physiological factors, or cold temperatures. Earlier studies, however, have indicated increased sensitivity to 1080 at extreme temperatures. House mice (*Mus musculus*), guinea pigs (*Cavia porcellus*), and brushtail possums (*Trichosurus vulpecula*) were more susceptible to 1080 intoxication at high or low temperatures, or both, than at moderate temperatures (Chenoweth 1950, McIlroy 1981, and Oliver and King 1983). Raccoons (*Procyon lotor*) showed increased sensitivity at high (23-37°C) temperatures and opossums (*Didelphus virginiana*) might have been more sensitive to 1080 at low temperatures (Eastland and Beasom 1986).

Age has been reported to influence 1080 effects in some species (McIlroy 1981; Hudson et al. 1984). Although we did not determine ages of our test birds, their average age in winter was probably greater than in summer. Spring reproduction would provide a greater proportion of immature birds in the summer population. Older magpies could be more resistant to 1080, but this is unknown.

Birds that survived a day beyond dosing had lower tissue residues compared to birds that died soon after treatment. This result indicates that magpies metabolize 1080 rapidly, and suggests that potential nontarget hazard from magpies that ingest 1080 quickly decreases with increased survival time. No sex differences in sensitivity to 1080 were apparent among magpies.

The presence or absence of 1080 residue in tissue or stomach samples has sometimes been used to indicate that animals did, or did not, die from 1080 intoxication. In our tests, 1 of 76 birds (1.3%) that consumed 1080 showed no tissue residue. Also, 1080 residues were found in birds that remained alive, and probably would have survived, but were euthanized 24 h after ingesting 1080. This finding suggests that it would be possible to collect a live magpie that had ingested 1080 and showed residue, but that might not have died of 1080 intoxication. The finding also indicates that detecting 1080 residue in magpies that died of unknown causes does not establish unequivocally that 1080 caused the death. Hence, using the presence or absence of 1080 residue as an indication of death from 1080 intoxication among magpies could occasionally be erroneous.

LC_{50} Tests

Magpies fed diets containing nominal concentrations of 5-20 ppm 1080 apparently lost weight but showed little reduction in food consumption. This was consistent with reports on some other species. European ferrets (*Mustella putorius*) and mink (*Mustella vision*) fed 1080 treated diets lost weight with little reduction in food intake (Hornshaw et al. 1986). The researchers suggested that 1080 affected weight by interfering with metabolism rather than food consumption. However, magpies at higher doses reduced consumption and lost weight. Burns et al. (1991) observed that skunks (*Mephitis mephitis*) and golden eagles also reduced daily intake of 1080-treated diets. The effect of consuming 1080-treated diets apparently differs among species and dose levels.

Hornshaw et al. (1986) noted that the threshold of daily 1080 consumption in treated diets for ferrets and mink, without deaths, approximated their LD_{50}s. We found no such relationship in magpies. At the LC_{50} (16 ppm) magpies ate about 0.70 mg/bird/day. For a bird weighing 160 g this equals 4.2 mg/kg, or about twice the LD_{50} (2.12 mg/kg).

Our work indicated that 1080 was less toxic to magpies than previously reported. Among birds killed by 1080, residue was detected in muscle or gizzard-stomach after death in about 99%, indicating *postmortem* residue analysis is useful in determining 1080 intoxication. However, such analyses might not be definitive; a bird in our studies that ingested 1080 showed no detectable 1080 residue and 1080 residue was detected in all birds euthanized 24 h after surviving a "mid-level" 1080 dose.

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Table 4. Body weights (g), food consumed (g/bird/day), and 1080 consumed (mg/bird/day[a]) during 5-day LC_{50} test of Compound 1080 with magpies.

Dose/parameter	Acclimation period (mean ± sd)	Test period (mean ± sd)	Test period change	Posttest period (mean ± sd)	Posttest period change
Dose rate = 0.0 (n = 12[b])					
Bird weight	164.4 ± 12.6	168.6 ± 13.2	+ 4.2	165.1 ± 14.8	− 3.5
Food eaten	40.3 ± 15.2	46.4 ± 12.7	+ 6.1	42.5 ± 11.8	− 3.9
1080 ingested		0.00			
Dose rate = 2.5 (n = 6)					
Bird weight	159.5 ± 6.2	169.0 ± 4.4	+ 9.5	171.7 ± 9.6	+ 2.7
Food eaten	48.0 ± 15.4	51.0 ± 13.1	+ 3.0	45.9 ± 10.8	− 5.1
1080 ingested		0.13			
Dose rate = 5.0 (n = 6)					
Bird weight	174.2 ± 18.0	173.3 ± 15.3	− 0.9	177.5 ± 9.8	+ 4.2
Food eaten	47.8 ± 13.5	44.4 ± 17.2	− 3.4	45.3 ± 15.2	+ 0.9
1080 ingested		0.22			
Dose rate = 10.0 (n = 6)					
Bird weight	151.0 ± 4.7	133.3 ± 10.2	−17.7	144.4 ± 13.3	+ 11.2
Food eaten	40.3 ± 15.5	42.7 ± 19.1	+ 2.4	38.6 ± 17.9	− 4.1
1080 ingested		0.43			
Dose rate = 20.0[c] (n = 6)					
Bird weight	159.7 ± 18.1	134.0 ± 8.7	−25.7	140.2 ± 11.4 [d]	+ 6.2
Food eaten	45.7 ± 15.6	40.3 ± 18.2	− 5.4	40.3 ± 12.8	0.0
1080 ingested		0.83			
Dose rate = 40.0 (n = 6)					
Bird weight	151.3 ± 6.1	139.8 ± 20.5	−11.5	(No survivors)	
Food eaten	39.5 ± 16.1	14.3 ± 8.0	−25.2		
1080 ingested		0.57			
Dose rate = 80.0 (n = 6)					
Bird weight	158.2 ± 25.4	152.0 ± 23.4	− 6.2	(No survivors)	
Food eaten	36.7 ± 16.7	8.9 ± 6.6	−27.8		
1080 ingested		0.72			

[a]Based on 90% ai for Compound 1080. Subsequent analysis indicated 94.5% ai.
[b]6 birds each in tests 1 and 2.
[c]5 birds in Test I plus 1 bird in Test II.
[d]For posttest, n = 4 (2 birds died).

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