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## SECONDARY POISONING OF OWLS BY ANTICOAGULANT RODENTICIDES

4/26/79

In a phone call, Lucille Strickel gave permission for us to release the results of this study to the registrant. The study has been/is going to shortly be submitted for publication. (was already rejected by Science)

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rodent control poisons that kill by causing hemorrhage are widely used, and avian predators may capture poisoned rodents. Owls were fed rodents killed with six anticoagulant baits. Four compounds led to hemorrhaging in some owls; hemorrhaging was lethal with bromodiolone, brodifacoum, and diphacinone, and sublethal with difenacoum. No adverse effects occurred with fumaarin and chlorophacinone.

anticoagulants (1) constitute over 90% of all rodent poisons used in the United States (2). The compounds cause hemorrhage by blocking synthesis of clotting factors at a step involving vitamin K (3). Several days are normally required after initial ingestion of the anticoagulant before stores of factors are exhausted and symptoms appear. The delayed action allows poisoned rodents to remain active and available to predators for several days. This poses a potential hazard of secondary poisoning, in which predators ingest toxicants that are present in tissues of their prey rather than by direct intake. Miscellaneous accounts of such poisoning include a cat and possibly skunks that died from eating prey containing warfarin, rats from eating prey containing difenacoum, and mink and dogs from eating prey containing five different compounds (1,4,5). Dogs, however, were not affected by warfarin-poisoned mice (6). Anticoagulant secondary poisoning among birds or prey has not been reported, and was examined during the present study.

In a preliminary trial conducted in 1970 at Olympia, Washington, three great-horned owls (Bubo virginianus) and one saw-whet owl (Negolius acadicus) were each fed two diphacinone-killed mice (Peromyscus maniculatus) daily for 5 days. Mice had consumed a lethal dose of toxicant during a 10-day free-choice bioassay. Each mouse was fed one g daily of an oat-groat bait containing 0.01% diphacinone (7), and individual bait consumption was recorded daily; unadorned Purina Lab Chow<sup>®</sup> was also available ad libitum. Owls were fed two poisoned mice daily for five days, followed by chicken heads on each test day and during a subsequent 20-day observation period. Coagulation was measured in all owls on days 0 (pre-treatment), 6, and in one great-horned owl, on days 15 and 22. Blood (0.1 cc or less) was collected from the brachial vein in a non-heparinized microhematocrit tube, and was teased with a hooked needle until the first strand of fibrin appeared. This

provided an index of coagulation time that was reproducible to within 1 min for normal coagulation, a sensitivity sufficient for assessing anticoagulant intoxication. Normal times were approximately 2.0 min.

In the principal experiment, 30 barn owls were fed rats (Rattus norvegicus, R. rattus, and R. exulans) captured in Niho, Hawaii, and poisoned with diphacinone, chlorophacinone, fumarin, difenacoum, bromadiolone, or brodifacoum (1). Individually caged rats were fed oat-grout baits containing registered or recommended concentrations of toxicant: 0.025% fumarin, 0.002% brodifacoum, and 0.005% other compounds. Baits were fed for 5 days on a free-choice basis (Lab Chow was available as before), and bait consumption was recorded daily. Anticoagulant-killed rats were fed to owls for periods of 1, 3, 6, or 10 days to allow comparison of various periods of exposure that seemed likely to be encountered in the field. Undosed rats were fed to the owls each afternoon; portions not eaten were weighed and recorded the next morning, including an estimate of the fractions of alimentary tract (containing possible unabsorbed toxicant) and of liver (containing the majority of absorbed toxicant and metabolites) (5). The experiment was run in three parts: 1., feeding of dosed rats for 1 and 6 days; 2., feeding of dosed rats for 3 and 10 days; and 3., replicate of (2). In each part there was one owl per toxicant for each feeding regime plus two controls. Species of rats fed to each owl were mixed, except that all in part 3 were R. exulans.

Owls were obtained from the breeding colony at Patuxent Wildlife Research Center and were housed during the experiment in individual indoor cages measuring 55 X 75 X 61 cm. The daily light regime in parts 1 and 2 was 13L:11D, but in part 3 it was 10L:14D (to inhibit possible development of the gonads, since the month was March). During "dark" hours, a hooded 7 1/2 w

bulb provided slight illumination. Pre-experimental weights ranged from 425 to 605 g (Table 2); post-treatment weights had not changed significantly.

Coagulation indices were measured 8 days before dosing, 20 days after first dosing, and (part 2 only) on the 3rd day after the end of dosing. Pre-test coagulation times ranged from 0.25 to 3.35 min. Birds that died during the experiment were necropsied on the day of death; owls that survived to day 20 were sacrificed with chloroform and necropsied.

All four owls in the preliminary trial displayed anticoagulant poisoning, and three died from massive hemorrhaging (Table 1). Coagulation indices on day 0 were elevated by 22-34+ min; in the survivor, recovery was only partial by day 10 (6 min).

In the principal experiment, six barn owls died: five that were fed brodifacoum rats, and one fed bromadiolone rats (Table 2). Birds fed difenacoum survived, but those on 0- and 10-day feeding regimes hemorrhaged (Table 2), one of them (no. 413) severely. Other birds fed bromadiolone, and all those fed rats poisoned with diphacinone, fumarin, and chlorophacinone survived without apparent intoxication.

Hemorrhages occurred throughout the carcass, including subcutaneous areas and visceral organs. Sublethal lesions were similar to lethal ones, but were less numerous and severe. Only dead birds, however, displayed hemorrhage of the heart wall or distension of the pericardium by clear or bloody fluid. The coagulation index was elevated (10 min) in the bird sampled shortly before death (no. 57); other coagulation indices were within normal range.

We have demonstrated a potential hazard to avian predators of secondary poisoning by four anticoagulant rodenticides. brodifacoum and bromadiolone were lethal to some barn owls, and difenacoum produced

sublethal hemorrhaging in this species at the levels tested. The only compound tested on three species of owls, diphacinone, was toxic to great-horned and saw-whet owls during the preliminary trial, but not to barn owls in the principal experiment. Possible explanations include interspecific differences in susceptibility among the owls, or differences in prey species and hence in metabolites presented to them (8). However, we can draw no conclusions without further comparative tests using a consistent protocol.

The effects of anticoagulants on raptors in the field remain to be assessed. The amount of toxicant ingested by rodents under natural conditions is probably similar to that under our regime (free-choice bioassay of each bait at registered or recommended concentrations). A large-scale control program also exposes predators to poisoned prey for a number of days. Therefore, secondary poisoning of raptors by anticoagulants under certain conditions seems likely. The effect of a given dose on birds may be exacerbated by conditions in the field, including stress, changes in diet (9) or increased activity (10). Minor injury can also increase susceptibility, even if the injury has occurred before exposure (owl no. 253 suffered massive hemorrhage at the site where blood had been sampled 17 days earlier). Caution is indicated in the use of anticoagulants for rodent control unless toxicity tests have shown that little danger exists for the combinations of compound, predator, and prey species concerned. This is of particular concern where avian predators on rodents are rare or endangered.

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1. Anticoagulants discussed include 4 registered as rodenticides in the U. S.: warfarin (3-[ $\alpha$ -acetylbenzyl-4-hydroxycoumarin], diphacinone [2-(diphenylacetyl)-1,3-indandione], chlorophacinone (2-[(p-chlorophenyl)phenylacetyl]-1,3-indandione), fumarin [3-( $\alpha$ -acetylfurfuryl)-4-hydroxycoumarin]; and 3 experimental ones: difenacoum [3-(3-p-diphenyl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin], brodifacoum (3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin), and bromadiolone (3-[3-(4'-bromobiphenyl-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxycoumarin). Use of trade names does not imply endorsement of commercial products by the Federal government.
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Table 1. Secondary toxicity of diphacinone to owls.

| Species  | Owl        | Mice fed to owls |               | Days        |
|----------|------------|------------------|---------------|-------------|
|          | wt.<br>(g) | Total<br>wt. (g) | Dose<br>(mg)* | to<br>death |
| Great-   | 1271       | 175              | 5.5           | --          |
| horned   | 1226       | 156              | 4.1           | 14          |
|          | 1135       | 143              | 4.6           | 14          |
| Saw-whet | 110        | 156              | 6.1           | 7           |

\* Total toxicant consumed by mice.

Table 2. Secondary toxicity of six anticoagulants to barn owls (Tyto alba). The full range of doses is shown for the first 3 toxicants; for the last 3 (no effect), only the maximum dose.

| Toxicant          | Days dosed | Owls |         | Rats offered |                        | Rats eaten  |        | Intox. |              |       |
|-------------------|------------|------|---------|--------------|------------------------|-------------|--------|--------|--------------|-------|
|                   |            | Bird | Wt. (g) | Total wt(g)  | Dose (mg) <sup>+</sup> | Total wt(g) | Livers |        | Intes- tines |       |
| Difena-<br>coum   | 1          | 298  | 495     | M            | 72                     | 1.74        | 66     | 1      | 1/4          | --    |
|                   | 3          | 393  | 430     | M            | 336                    | 6.42        | 270    | 3      | 2 3/4        | --    |
|                   | 3          | 70   | 480     | F            | 189                    | 4.54        | 125    | 2 1/4  | 3            | --    |
| Broma-<br>diolone | 6          | 246  | 495     | M            | 586                    | 9.81        | 174    | 1 1/6  | 2 1/2        | H     |
|                   | 10         | 311  | 510     | F            | 1160                   | 12.54       | 567    | 4 2/3  | 5 5/8        | H     |
|                   | 10         | 413  | 540     | F            | 595                    | 7.99        | 477    | 10     | 5 7/8        | H     |
| diolone           | 1          | 306  | 460     | M            | 118                    | 2.65        | 52     | 1      | 7/8          | --    |
|                   | 3          | 374  | 450     | M            | 358                    | 6.60        | 281    | 3      | 3            | --    |
|                   | 3          | 52   | 425     | M            | 228                    | 3.96        | 146    | 3      | 2 3/4        | --    |
| diolone           | 6          | 401  | 490     | M            | 625                    | 11.11       | 295    | 5      | 4            | --    |
|                   | 10         | 258  | 540     | F            | 1106                   | 14.59       | 576    | 7 5/6  | 4 1/2        | --    |
|                   | 10         | 68   | 635     | F            | 710                    | 9.63        | 463    | 8 1/2  | 5 1/8        | D(11) |

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Table 2. (continued)

| Toxicant             | Days dosed | Owls |         | Rats offered |             | Rats eaten             |             | Intox. signs* |        |              |
|----------------------|------------|------|---------|--------------|-------------|------------------------|-------------|---------------|--------|--------------|
|                      |            | Bird | Wt. (g) | Sex          | Total wt(g) | Dose (mg) <sup>+</sup> | Total wt(g) |               | Livers | Intox. signs |
| Brodifa-<br>coum     | 1          | 395  | 400     | M            | 71          | 0.58                   | 67          | 1             | 1/2    | --           |
|                      | 3          | 247  | 430     | M            | 400         | 2.50                   | 299         | 3             | 2 1/2  | D(8)         |
|                      | 3          | 57   | 475     | M            | 223         | 1.75                   | 154         | 3             | 1 3/8  | D(11)        |
|                      | 6          | 253  | 505     | F            | 580         | 3.84                   | 370         | 5 2/3         | 3 1/4  | D(9)         |
|                      | 10         | 403  | 470     | F            | 814         | 3.15                   | 492         | 6             | 4 7/8  | D(8)         |
| Dipha-<br>cinone     | 10         | 254  | 545     | F            | 558         | 3.30                   | 368         | 7             | 3 3/4  | D(8)         |
|                      | 10         | 416  | 485     | F            | 1195        | 11.69                  | 848         | 10            | 7 5/8  | --           |
| Fumarin              | 10         | 259  | 595     | F            | 575         | 9.04                   | 490         | 9 7/8         | 7      | --           |
|                      | 10         | 377  | 520     | F            | 1137        | 73.62                  | 751         | 10            | 7 3/8  | --           |
| Chloro-<br>phacinone | 10         | 66   | 595     | F            | 654         | 48.89                  | 605         | 10            | 8 5/8  | --           |
|                      | 10         | 329  | 475     | M            | 1276        | 16.07                  | 655         | 7 1/3         | 5 1/2  | --           |
|                      | 10         | 39   | 605     | F            | 712         | 9.16                   | 576         | 9             | 3 1/2  | --           |

+ Total toxicant consumed by rat. \* Signs of intoxication: -- = no signs; H = hemorrhage, survived;

D = hemorrhage and death (number gives day of death from start of dosing).