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LABORATORY EFFICACY STUDIES WITH STRYCHNINE BAITS ON PIGEONS

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ABSTRACT. Pigeons held under a fall and spring photoperiod-temperature regime consumed a maximum of 16.9 to 21.4 kernels of whole corn per hour with an average ranging from 7.6 to 12.4 kernels per hour. Peak consumption occurred during the first and/or next to last hour of the day with a secondary, but smaller, peak around noon. Corn consumption ranged from 91 to 112 kernels/bird/day and approximated daily consumption equal to 10% of an average bird's mass.

The acute oral LD_{50} of strychnine alkaloid to pigeons was estimated to be 7.73 mg/kg (95% Confidence Interval of 6.75 to 8.85). The LD_{90} was 10.99 mg/kg (8.79 to 13.7) and the LD_{10} was 5.37 mg/kg (4.35 to 6.63). Time to death ranged from 5 to 39 minutes. Treatment of groups of 10 pigeons at 6-hour intervals beginning at 0600h with 7.73 mg/kg strychnine suggested that pigeons were more sensitive to intoxication at 1200h and 1800h than they were at 0600h.

Laboratory tests of 0.2, 0.4 and 0.6% strychnine-treated whole corn bait with pigeons during the first hour of the day and at noon indicated that mortality from the 0.2% bait was insufficient for adequate control (17 to 33%). The 0.4% and 0.6% baits, however, produced acceptable mortality (48 to 71%). Pigeons killed by the 0.4% and 0.6% baits consumed from 11 to 21 kernels of corn depending upon the time of ingestion. Strychnine residues in the crop and gizzard contents of pigeons consuming 0.4% and 0.6% baits ranged from 455 ppm to 1500 ppm. Residues in birds fed the 0.4% baits were 37% that of those fed the 0.6% bait during the early morning, and 49% that of birds exposed at noon. Gizzard residues ranged from 16 ppm to 38 ppm and intestinal tissue residues ranged from 4.6 ppm to 9.2 ppm, and were not correlated with treatment or treatment time. Strychnine residues in organs (heart, liver, and kidney) ranged from not detectable to 2.2 ppm and residues in muscle from 0.17 ppm to 0.56 ppm.

These data indicate that a 0.4% strychnine alkaloid bait may be an effective substitute for the 0.6% bait now registered by the U.S. Fish and Wildlife Service (Pigeon Bait Poisoned Grain, Reg. No. 6704-42). The exposure of predatory and scavenger birds and mammals to potential secondary poisoning following the consumption of strychnine-treated pigeons could be reduced by 51% to 63% with the use of the 0.4% bait.

INTRODUCTION

In early 1984, the U.S. Environmental Protection Agency (EPA) notified the U.S. Fish and Wildlife Service (FWS) that a mistake was found in the calculations used to determine the strychnine alkaloid concentration in FWS registration 6704-42 (Pigeon Bait-Poisoned Grain). The mistake was found in data contained in a 1963 letter submitted by Mr. C. E. Faulkner to support the registration of the 0.6% strychnine concentration. These data were based on the English measurement system of ounces and quarts and summarized laboratory tests conducted at an unknown location with various concentrations of strychnine. Pilot studies initiated at the Denver Wildlife Research Center (DWRC) indicated that if the assumptions contained in Faulkner's letter were valid, the minimum effective strychnine concentration for pigeons (Columba livia) was 0.6%. These studies also indicated that additional data were needed to determine the correctness of Faulkner's assumptions.

Studies were initiated to determine: 1. The daily and hourly food consumption capabilities of pigeons held under two photoperiod and temperature conditions; 2. The acute oral toxicity of strychnine to pigeons; and 3. The potential diurnal sensitivity of pigeons to strychnine intoxication. These data were then used to determine: 1. If the registered 0.6% rate of strychnine could be lowered and still retain efficacy for pigeons; 2. If the treated bait consumption figure used by Faulkner (15 kernels) was realistic; and 3. What levels of strychnine residues resulted following the exposure of pigeons to strychnine-treated baits.

METHODS

Food Consumption

Five groups of 4 overnight fasted pigeons were monitored hourly from 0600h to 1800h (MST) to determine the number of whole corn kernels consumed by individual pigeons. Five additional groups were tested for the first 3 hours of the day (0600h to 0900h). All birds were acclimated to group-holding conditions ($(2m)^3$ wire mesh cages) for 1 week with average temperatures ranging from 21 to 25°C and a photoperiod of 12h light:12h dark. Two fasting periods were also used (12h and 16h) to determine if longer fasting periods would result in greater food consumption during the test period. In addition, 4 pairs of pigeons (mixed sex) were conditioned in $(0.5m)^3$ wire-mesh cages in a controlled environment chamber (Sherer Model CEL-37-14) at 5°C under a photoperiod of 9h light:15h dark for 2 weeks. At the end of this period they were tested for one 9h light cycle. In all tests, food consumption was determined by weighing the corn consumed during each hour and by converting these data to the average number of

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kernels consumed/bird. From these data, the maximum and minimum number of kernels consumed by pigeons for each hour, the average number consumed throughout the day, and the standard error of the mean (s_x) for each response was determined.

LD_{50} Determination

Technical grade strychnine alkaloid (100%) supplied by the FWS Pocatello Supply Depot (PSD) was formulated in propylene glycol. The acute oral LD_{50} was determined by gavage at lOOOh MST with fasted pigeons, using the methods described by Schafer et al. (1973) and a 7-day observation period. The test used from 6 to 10 pigeons at each of nine treatment levels, including a propylene glycol control. The LD_{10}, LD_{50}, and LD_{90} with 95% Confidence Intervals were calculated by probit analysis (Daum 1970, Finney 1971). In addition, three groups of 10 pigeons were gavaged with strychnine at the calculated LD_{50} level at three times of the day (MST: 0600h, 1200h, and 1800h) to determine if the time of ingestion influenced the toxicological responses.

Strychnine Bait Efficacy

Twelve groups of six pigeons were allocated to 4 replications of three treatments to determine the effectiveness of strychnine-treated whole corn in the laboratory, the average number of treated kernels consumed during periods of maximum food consumption, and residues in strychnine-killed pigeons. Each group was fasted for 12h to 16h and was offered 150 kernels (approximately six times the maximum average kernel consumption/h) from 0700h to 0800h (the peak feeding period) in communal wire-mesh cages measuring $(2m)^3$. Three levels of strychnine were prepared and used: 0.6%; 0.2% (the minimum effective level estimated from the LD_{50} and food consumption tests), and 0.4%, a level halfway between. These baits were prepared in 1 kg quantities using 0.75% Alcolec Lipoidol 211 as an adhesive and the procedures employed by the PSD for preparing the registered product. Strychnine was added to make the desired concentrations but the amount was increased by 6.5%, according to PSD practices, to make up for material lost in the vessels used to mix the baits. A similar study was also conducted with the treatments offered from 1200h to 1300h, the second peak feeding period.

The number of corn kernels remaining in the crop and gizzard of each pigeon killed during these tests were counted and frozen for residue analysis following a gross necropsy. Analysis was accomplished by thin layer chromatography (TLC) using 0.2mm-silica gel plates with fluorescent indicator (EM 5735). The plates were developed in a mobil phase of methanol and the solvent front was allowed to migrate approximately 15cm. Strychnine spots were visualized under 254nm ultraviolet light. The detection threshold ranged from 30 ppm to 37.5 ppm.

Gizzard, intestine, heart, liver, kidney, and muscle tissues of one randomly selected pigeon from each treatment group were collected and composited for each treatment group. These samples were analyzed for strychnine by high-pressure liquid chromatography (HPLC). A Spectra-Physics SP 8700 solvent delivery system and LDC 1201 Spectromonitor ultraviolet detector operated at 254nm were used to detect strychnine. The column was a Micro Pak MCN-10 octyldecylsilane reverse phase and was maintained at 24°C. The mobil phase consisted of 0.75% NH_4OH (v/v) in methanol at a flow rate of 1.5ml/minute. The detection threshold was 0.17 ppm.

RESULTS

Consumption of Untreated Corn

Pigeons held under a 12h photoperiod, 21 to 25°C temperature, and fasted for 16h, consumed the maximum number of corn kernels between 0600h and 0700h (the first hour of feeding), an average of 21.4 (s_x=5.2) kernels/bird/h (Figure 1). The average consumption throughout the 12h light cycle was 7.6 (s_x=2.2) kernels/bird/h. Peak consumption times were 0600h to 0700h and 1300h to 1400h, with kernel consumption during the latter time period being 13.4 (s_x=1.8) kernels/bird/h. Pigeons held under a 12h photoperiod but fasted for 12h also consumed the highest number of kernels during the 0600h to 0700h and 1300h to 1400h periods. The average number consumed during the first period was 16.9 (s_x=2.0) kernels/bird/h and during the second period was 11.6 (s_x=0.6) kernels/bird/h, while the average kernel consumption throughout the 12h day was 7.7 (s_x =1.0) kernels/bird/h.

Pigeons held under a 9h photoperiod at 5°C and fasted for 16h had a maximum consumption of 22.1 (s_x=3.4) kernels/bird/h which occurred between 1430h and 1530h, while a smaller feeding peak occurred from 0730h to 0830h at an average of 12.8 (s_x=1.3) kernels/bird/h. The average number of kernels consumed throughout the entire 9h photoperiod was 12.4 (s_x =1.3) kernels/bird/h.

The total number of kernels of untreated corn consumed on a daily basis for pigeons held under a 12h photoperiod ranged from 91 to 99 kernels/bird/d, depending upon the fasting period. The total number of kernels consumed by pigeons on a 9h photoperiod and 5°C was 112 (s_x =7.7)kernels/bird/d.

LD_{50} Determination

The acute oral LD_{50} (with 95% Confidence Intervals) of strychnine alkaloid to pigeons was estimated as 7.73 mg/kg (6.75 to 8.85) (Table 1). The LD_{10} value was estimated as 5.37 mg/kg (4.35 to 6.63) and the LD_{90} was estimated as 11.0 mg/kg (8.79 to 13.7). Death times ranged from 5 to 39 minutes. Symptomology was typical for strychnine poisoning and included tremors, salivation, and tonic and clonic convulsions. A 7.73 mg/kg dose administered to three groups of 10 pigeons indicated that they were less sensitive to strychnine at 0600h than they were 1200 and 1800h (Table 2).

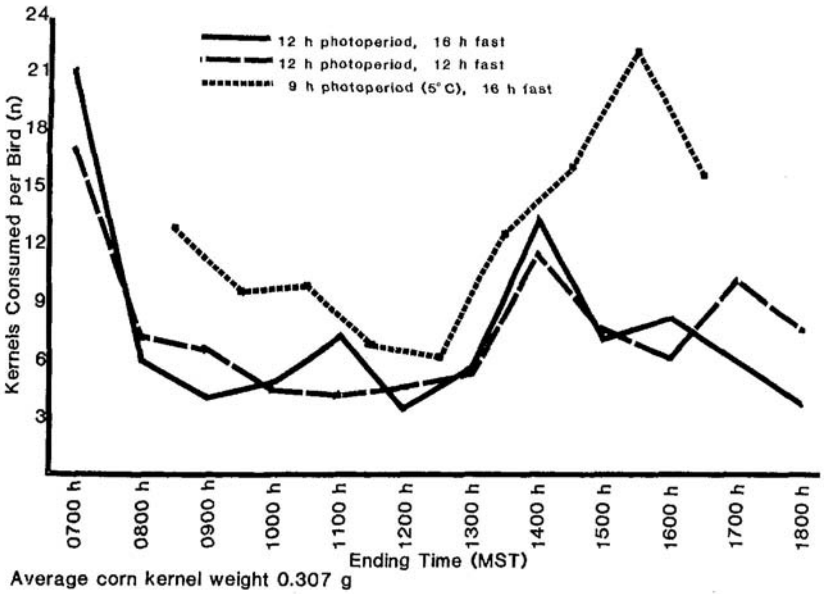

Figure 1. Hourly consumption of untreated corn by groups of pigeons held on 9h and 12h photoperiods and fasted for 12h or 16h.

Table 1. The acute oral toxicity of strychnine alkaloid to pigeons determined by propylene glycol gavage.

Dosage (mg/kg)	Mortality (no./ no. tested)	Death time (min)
31.6	6/6	5 - 15
23.7	5/6	6 - 36
17.8	6/6	6 - 22
13.3	6/6	10 - 31
10.0	8/10	12 - 39
7.50	4/10	8 - 16
5.62	2/10	20
4.22	0/10	-
Control	0/6	-

LD_{50} (95% CL) = 7.73 (6.75 to 8.85) mg/kg

LD_{90} (95% CL) = 11.0 (8.79 to 13.7) mg/kg

LD_{10} (95% CL) = 5.37 (4.35 to 6.63) mg/kg

Table 2. The acute oral toxicity of 7.73 mg/kg of strychnine alkaloid administered to pigeons in propylene glycol at 0600h, 1200h, and 1800h.

Time (MST)	Mortality (no./ no. tested)	Death time (min)
0600	1/10	20
1200	5/10	11 - 64
1800	5/10	20 - 48

Strychnine Bait Efficacy

Analyzed strychnine alkaloid levels in treated baits (HPLC) were within 4% of the target prepared levels, indicating that *very* little chemical was lost on vessel walls or through the treatment process (Table 3).

When 4 cages of 6 pigeons each were offered 150 corn kernels treated with 0.2% strychnine between 0600h and 0700h, 8 out of 24 birds (33%) were killed (Table 4). Gross necropsies found a total of 101 kernels crops and gizzards at this level (13 kernels/bird). We could not account for 101 kernels, indicating that the 16 surviving pigeons consumed an average of 7.4 kernels/bird without mortality. The average of 13 kernels found in dead pigeons contained a dose of 23 mg/kg strychnine, while the kernels apparently consumed by survivors contained 13 mg/kg. These data indicate that ingestion of approximately 2 LD_{90} doses of strychnine on whole corn produced mortality in most pigeons while the ingestion of a

Table 3. Prepared and analyzed residue levels of strychnine baits.

Concentration (%)	Prepared concentration (%)	Analyzed concentration (%)
0.20	0.21	0.21
0.40	0.43	0.45
0.60	0.64	0.63

single LD_{90} dose produced only transient nonlethal effects. Residues of strychnine in the crop and gizzards of dead pigeons averaged 150 ppm (Table 5). No other tissues were analyzed for this treatment level.

Table 4. Summary of kernel consumption and mortality of pigeons allowed to feed on 150 kernels of strychnine treated corn in the morning or afternoon.

Strychnine concentration (AM/PM)	Mortality (%)	# Kernels in cage	Average # kernels in dead pigeons	Average # missing kernels per live bird
0.2% AM	33	95	13	7.4
0.4% AM	58	83	15	7.2
0.6% AM	71	76	15	7.6
0.2% PM	17	108	19	22
0.4% PM	63	63	21	7.5
0.6% PM	42	114	11	9.5

Table 5. Residues of strychnine alkaloid in the gastrointestinal tract contents and tissues of pigeons killed by ingesting strychnine treated baits.

Concentration (%)	Crop & gizzard contents	Strychnine residues (ppm) Gizzard	Intestine	Liver	Kidney	Heart	Muscle
0.2 AM	150*						
0.4 AM	557	38	4.6	0.92	0.38	0.34	0.56
0.6 AM	1500*	26	8.7	1.2	ND*	1.8	0.17
0.2 PM	175						
0.4 PM	455*	16	9.2	1.3	0.56	1.3	0.34
0.6 PM	935*	27	6.5	0.95	2.2	0.51	0.18

* Does not include values designated as not detectable (ND).

With 0.4% strychnine baits, 14 birds were killed (58%), gross necropsies found 214 kernels in the crops and gizzards (15 kernels/bird) and 70 kernels (7.2 kernels/bird) were not accounted for(Table 4). The average of 15 kernels found in dead pigeons contained a dose of 51 mg/kg strychnine, or about 5 LD_{90} doses. The kernels apparently consumed by survivors contained an average dose of 25 mg/kg, or 2 LD_{90} doses without apparent lethal effects. Residues of strychnine averaged 557 ppm in the crop and gizzard contents (Table 5) while intestinal tissue averaged 4.6 ppm and gizzard tissue 38 ppm. Other organ and muscle tissue residues ranged from 0.34 ppm in the heart to 0.92 ppm in the liver (Table 5).

With the 0.6% strychnine bait, 17 birds were killed (71%), gross necropsies found 245 kernels in the crops and gizzards (15 kernels/bird) and 53 kernels (7.6 kernels/bird) were assumed to have been consumed by survivors (Table 4). The 15 kernels found in the dead pigeons contained an average dose of 79 mg/kg, or 7 LD_{90} doses. The kernels apparently consumed by survivors contained 40 mg/kg, or 4 LD_{90} doses. Residues of strychnine averaged 1500 ppm in the crop and gizzard contents (Table 5) while intestinel tissue averaged 8.7 ppm and gizzard tissue averaged 26 ppm. Organ and muscle tissue residues ranged from not detectable in the kidney to 1.8 ppm in the heart (Table 5).

When four cages of six pigeons each were offered 150 corn kernels treated with 0.2% strychnine during the 1200h to 1300h period, 4 out of 24 birds (17%) were killed (Table 4). Gross necropsies found

74 kernels in the crops and gizzards at this level (19 kernels/bird). Estimates of the actual number of treated kernels ingested or the average strychnine dose could not be made because some of the kernels represented previously consumed untreated corn. Similarly, the number of kernels consumed by surviving pigeons could not be calculated. Residues of strychnine present in the crop and gizzards of these birds averaged 175 ppm (Table 5). No other tissues were analyzed for this treatment level.

With the 0.4% strychnine bait, 15 birds were killed (63%), and gross necropsies found 338 kernels in the crops and gizzards (21 kernels/bird, Table 4). Strychnine residues averaged 455 ppm in the crop and gizzard contents (Table 5) while intestinal tissue averaged 9.2 ppm and gizzard tissue averaged 16 ppm. Organ and muscle tissue residues ranged from 0.34 ppm in the muscle to 1.3 ppm in the liver and heart (Table 5).

With the 0.6% strychnine bait, 23 of 48 birds were killed (48%), and gross necropsies found 213 kernels in the crops and gizzards (11 kernels/bird, Table 4). Residues of strychnine averaged 935 ppm in the crop and gizzard contents (Table 5) while intestinal tissue averaged 6.5 ppm and gizzard tissue averaged 27 ppm. Organ and muscle tissue residues ranged from 0.18 ppm in the muscle to 2.2 ppm in the kidney (Table 5). Three deaths occurred between 6 and 24 hours after bait exposure and these birds were not collected or analyzed for strychnine.

DISCUSSION

The average daily consumption of untreated corn by pigeons was uniform for birds held and fed under a 12h photoperiod at room temperature, irrespective of whether they were fasted for 12h or 16h. Consumption ranged from 91 to 94 kernels/bird/d (28 to 29 g/bird/d), or approximately 10% of each individual bird's mass. Peak feeding times occurred during the first hour of the morning and from 1200h to 1300h. Total daily consumption of pigeons held under a 9h photoperiod and at 5°C averaged 112 kernels/bird/d (28 g/bird/d), similar to the birds held under a 12h photoperiod.

The pattern of feeding was different for pigeons held under the 9h photoperiod, but could have been due to the environmental chamber which was used to expose pigeons to the reduced temperature and photoperiod regime. The peak feeding time for these pigeons was from 1430h to 1530h with a second peak during the first hour in the morning. Because of the 9h photoperiod, hourly consumption of corn for these birds was 33% higher than for birds held under the 12h photoperiod.

The 7.73 mg/kg LD_{50} for strychnine alkaloid on pigeons was one-third the value of 21.3 mg/kg reported by Hudson et al. (1984). However, this difference could be due to the use of geletin capsules as a carrier by Hudson et al. and the use of propylene glycol for our data. Flury, cited in Spector (1956), determined the lethal dose (LD) for strychnine (not known if it was the alkaloid or a salt) as 8.5 to 11.5 mg/kg for pigeons.

Results of the diurnal sensitivity test with strychnine provided some evidence that pigeons treated at 0600h were less sensitive than birds treated at 1200h or 1800h. Tests with the acute neurotoxin 4-aminopyridine, which is similar to strychnine in mode of action, indicated little effect of time of day on sensitivity (Tharwat 1984). Hu et al. (1970) and Piepho and Friedman (1977) present conflicting data as to whether diurnal differences in sensitivity to strychnine exist in mammals. Hu et al. showed that rats had a higher sensitivity during darkness with a peak at OlOOh. Piepho and Friedman showed that mice were more sensitive to strychnine intoxication during light periods, with a peak sensitivity at 1800h.

When cages of six pigeons each were offered 150 kernels of strychnine-treated corn at 0.2, 0.4 and 0.6% for one hour beginning at 0600h, mortality was related to increasing concentration. At 0.2%, 33% of the birds tested died, whereas at 0.4% and 0.6% the mortality rates were 58% and 71%, respectively. The test design that was used for this evaluation was specifically selected to produce intermediate levels of mortality at all treatment levels so that the effects of different treatment rates could be analyzed. Mortality of pigeons offered treated whole corn during the second feeding period from 1200h to 1300h was more variable, with the 0.4% level showing the highest level of mortality (63%), followed by the 0.6% level at 48% and the 0.2% level at 17%. Because birds tested at this time period had been feeding for 6h and were under considerably less stress to consume treated corn baits, it is conceivable that the lower mortality rate for the 0.6% birds represents either repellency or conditioned aversion to the strychnine baits. Strychnine alkaloid has been shown to be highly repellent to redwinged blackbirds (Schafer et al. 1983), with an R50 (similar to an LD5Q) of 0.030%. Ward and Munch (1930) showed that rats can detect and avoid water treated with 5.4 ppb strychnine; however, Hartley (1975) concluded that strychnine was not capable of causing illness-induced aversion in rats responding to the taste of succrose.

The consumption of strychnine-treated corn during the morning or afternoon was uniform and independent of the strychnine concentration present. Consumption of strychnine-treated kernels averaged almost 15 kernels/bird, the same figure quoted by Faulkner in his 1963 letter to EPA. Of great interest is the fact that surviving birds consumed a uniform number of baits without mortality, averaging 7 kernels/bird for all three concentrations. Based on these data, pigeons may be detecting the presence of strychnine by a physiological means allowing a number of birds to survive. It is difficult, however, to reconcile the number of LD_{90} doses consumed by survivors (up to 4) with the lack of mortality, unless pigeons monitor their corn consumption allowing them to become sublethally intoxicated over time and to maintain that level with small additional amounts of treated bait. We have observed this phenomenon in pigeons and ducks fed psychoactive drugs.

Residues of strychnine in the crop and gizzard contents of pigeons dying following early morning ingestion of treated baits increased from 150 ppm for birds fed 0.2% baits, to 557 ppm for those fed 0.4% baits, and to 1500 ppm for pigeons fed 0.6% baits. Residue levels in the gizzard and intestine were not related to treatments and ranged from 4.6 ppm to 38 ppm. Organ and muscle tissue residues ranged from 0.17 ppm to 1.8 ppm, also with little apparent relationship to treatments.

For pigeons treated with 0.4% strychnine, the average dead bird ingested 51 mg/kg or 15 mg of strychnine. The maximum amount of strychnine recovered from the crop and gizzard constituted 2.1 mg or only 14% of the amount of strychnine ingested. Residues of strychnine in the remaining tissues that were analyzed accounted for less than 1.5 mg of strychnine, or a total of 3.6 mg. This represents 24% of the ingested amount. Similar calculations for the 0.6% bait with 79 mg/kg of ingested strychnine (22.5 mg), accounted for a total of 7.0 mg of strychnine. Thus, for pigeons treated with 0.6% strychnine baits, 31% of the ingested strychnine was found in the tissue and gut contents. The fate of the remaining strychnine is not known but must be associated with metabolism and excretion (Redig et al. 1982). No known studies have addressed the rate of strychnine metabolism in pigeons, nor the metabolic products.

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