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Retention of Physiological Marks by Coyotes Ingesting Baits Containing Iophenoxic Acid, Mirex, and Rhodamine B

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ABSTRACT: Pen-reared coyotes (*Canis latrans*) voluntarily ingested small tallow baits containing 10 or 15 mg of iophenoxic acid, 100-mg mirex, and 150-mg rhodamine B. One week after treatment with 10 and 15-mg iophenoxic acid, mean serum iodine was elevated from pretreatment levels of less than 5 $\mu\text{g}/100\text{ mL}$ to 175 and 324 $\mu\text{g}/100\text{ mL}$, respectively. Serum iodine levels declined to about 25 $\mu\text{g}/100\text{ mL}$ after four months, but were still fivefold higher than controls. Coyotes that ate baits containing 100-mg mirex averaged 0.34-ppm mirex in serum one week later levels declined to 0.15 ppm after four months. Serum samples obtained 2 and 24 h after death contained higher concentrations of iodine and mirex than samples obtained immediately before death. Although some back guard hairs of coyotes fed 150-mg rhodamine B showed distinct fluorescent bands, rhodamine B did not appear to provide a dependable mark because all guard hairs were not marked.

KEY WORDS: coyote, fluorescence, iodine, iophenoxic acid, mirex, physiological markers, rhodamine B

Oral marking agents (physiological markers) can be useful for comparing the efficacy of different baiting systems for delivering materials (that is, toxicants, vaccines, etc.) to free-ranging wild animals, provided: (1) they do not deter animals from ingesting the bait material; (2) the "marks" are detectable; and (3) the "marks" persist long enough to meet the requirements of the study. Our interest in comparing the efficacy of several small bait placement strategies on coyotes suggested a need for several oral marking agents. Linhart and Kennelly [1] demonstrated the utility of demethylchlortetracycline (demeclocycline; 7-chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1, 11-dioxo-2-naphthacene-carboxamide) as a long-term physiological marker. Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta[cd]pentalene) and iophenoxic acid (α -ethyl-3-hy-

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droxy-2,4,6-triiodobenzeneproanoic acid) have also proven useful [2,3], the former because it can be detected in blood, muscle, and fat for long periods of time and the latter because it causes greatly elevated iodine levels in blood for extended periods [4]. Larson et al. [2] used iophenoxic acid (IA) and mirex to identify bait-ingesting coyotes for eight weeks, while Baer et al. [3] reported elevated iodine levels from IA for 34 and 52 weeks in red fox (*Vulpes vulpes*) and dogs (*Canis familiaris*), respectively. Rhodamine B (N-[9-(2-carboxyphenyl)-6-(diethylamino)-3H-xanthen-3-ylidene]-N-ethylethanaminium chloride) also has shown some promise as a physiological marker by creating fluorescent bands in the hair and toenails of mammals, including coyotes [5,6].

Since our projected field studies required recognition of coyotes four months after eating baits containing mirex, IA, and rhodamine B, tests over longer periods than previously reported, and specifically applicable to coyotes, were warranted.

Procedures

We selected twelve adult coyotes, six males and six females. All had been raised in captivity at the Millville (Utah) Predator Research Facility and were born of parents two or more generations removed from wild stock. During the study coyotes were maintained individually in adjacent 1.3 by 4 by 2-m chain-link kennels. Six days per week they were fed 700 g of a commercial ration prepared locally for the fur-farming industry. Water was available ad libitum.

Three treatment groups of four coyotes each (two males and two females) were established. All three groups were prebaited (one bait/coyote/day) for three days with untreated 9-g melted and molded beef tallow baits. Treatments were effected 5 to 7 Sept., followed by a four-month posttreatment period which ended on Jan. 3. During the three-day treatment period, all animals received one tallow bait per day. The treatment regimen is shown in Table 1.

On the day before treatment and during Weeks 1, 4, 8, 12, and 16 after treatment, the coyotes were immobilized with a mixture of 100-mg ketamine hydrochloride and 1-mg acepromazine administered intramuscularly. Two 15-mL blood samples were drawn from the saphenous vein(s), and hairs of the tail and ears as well as vibrissae and toenails were examined under a 366-nm ultraviolet (UV) lamp for evidence of fluorescence. A swatch of guard hairs, including the hair follicles, was removed from the back of each animal and also examined under the UV lamp. The blood samples, one heparinized and the other clotted, were subsequently centrifuged and plasma and sera, respectively, aspirated, stored in vials, and kept frozen until analyses were performed.

Following sampling at Week 16, the anesthetized coyotes were killed by suffocation. Two hours after death a blood sample was extracted from the vena cava, centrifuged, and the supernatant fluid removed and frozen. Twenty-four hours after death a sample of clotted blood was expressed from the carotid artery and/or heart and centrifuged to provide a second postmortem serum sample. A 20 to 25-g sample of hip muscle was removed at this time, placed in a clean self-sealing plastic bag, labeled, and frozen. All sample analyses for iodine, mirex, and rho-

TABLE 1—Treatment regimen for the three experimental groups of coyotes.

Group No.	Treatment Day		
	1	2	3
Group I	placebo	placebo	placebo
Group II	100-mg mirex	10-mg IA	150-mg rhodamine B
Group III	100-mg mirex	15-mg IA	150-mg rhodamine B

damine B were conducted "blind" (that is, personnel conducting the analyses and examinations had no prior knowledge of the treatment regimen).

Analytical Techniques for Iodine and Mirex

Reagents to measure iodine in plasma or sera were purchased from a commercial source [7], and analyses were conducted according to their method. A general synopsis of this method for determining total iodine is as follows: Pipette 0.05 mL (50 μ L) of each standard (0.0, 5, 10, 15, and 20- μ g iodine/100 mL) and unknown sample into an appropriately marked test tube. Add 1 mL of Digestion Reagent to each test tube. Starting with the first standard tube and at timed 5-s intervals, place each tube in a 230°C \pm 5°C preheated heating block in a hood. Exactly 6 min after placing the first tube in the heating block, and in the same timed 5-s intervals, remove the tubes and place in a rack in a hood to cool to room temperature. When cool, add 1 mL of Ceric Reagent to each test tube, mix well, and place in a rack in a 37°C water bath to equilibrate. Beginning with the first tube, and at timed 15-s intervals, remove each tube from the water bath and add 1 mL of Arsenious Reagent. Mix well and return tube to the water bath. Exactly 20 min after the Arsenious Reagent was added to the first tube, and at 15-s intervals, remove the test tube from the water bath and mix by swirling. The absorbance is measured against deionized water in a spectrophotometer at a wavelength setting of about 420 nm, such that the absorbance of the 0.0 mg I/100 mL standard is approximately 0.7. Composition of the reagents as stated by the manufacturer [7] were as follows: Digestion—(w/v): 0.05% ammonium tavanadate in 72% perchloric acid; Ceric—(w/w): 0.6% ceric ammonium sulfate in 27% sulfuric acid; Arsenious—(w/w): 0.9% arsenic trioxide in 8.3% sulfuric acid plus (w/w) 0.18% sodium chloride.

Standards were run every time samples were analyzed and a standard curve was made by plotting the absorbance of each standard (ordinate) against its concentration (abscissa). Values for the unknown samples were obtained directly from the curve. If any unknown sample had a value greater than the highest standard value, it was diluted with a known volume of distilled water and the procedure was repeated. The dilution value was used for determining total concentration of iodine.

The mirex in plasma, sera, or muscle tissue was analyzed by gas chromatography according to procedures used to determine organochlorines in biological samples [8]. We used 1-g samples of either plasma or sera and 10-g samples of muscle for analysis. The detection limit was 0.02 ppm.

Results

Iophenoxic Acid

The mean iodine levels of the three treatment groups during the pretreatment period ranged from 2.3 to 4.6 μ g/100 mL (Table 2, Fig. 1). During posttreatment, the mean iodine in the control group ranged from 2.1 to 3.2 μ g/100 mL. The two treatment groups receiving IA showed marked increases in blood iodine levels. Among subjects receiving 10 mg of iophenoxic acid, iodine levels rose to a mean of 175 μ g/100 mL (range = 143 to 206) the week following treatment. Levels started declining four weeks after treatment and continued to do so until the end of the study when a mean of 25 μ g/100 mL (range = 13 to 33) was recorded, nearly fivefold higher than controls. Within the 15-mg treatment group, blood iodine levels rose higher (\bar{x} = 324 μ g/100 mL, range = 246 to 420) than the 10-mg treatment group the week following treatment and also started to decline after four weeks, approximating levels of the 10-mg treatment group by 16 weeks posttreatment. Curiously, blood iodine levels in samples from both IA treatment groups were elevated two to three times among samples taken 2 and 24 h after death compared to those taken immediately prior to death. Within specific sampling periods and

TABLE 2—Concentrations of iodine [$\mu\text{g}/100 \text{ mL} \pm$ standard error (SE)] in coyote sera.

Time	Treatments (n = 4 per Group)		
	Control	10-mg IA ^a	15-mg IA
Pretreatment	2.5 \pm 1.1	2.3 \pm 0.7	4.6 \pm 0.3
Posttreatment			
1 week	3.2 \pm 0.4	175 \pm 14	324 \pm 40
4 weeks	2.1 \pm 0.2	169 \pm 31	350 \pm 42
8 weeks	2.7 \pm 0.5	101 \pm 18	246 \pm 65
12 weeks	2.6 \pm 0.4	44 \pm 10	112 \pm 17
16 weeks	2.7 \pm 0.4	25 \pm 4.5	29 \pm 4.0
Postdeath			
2 h	...	88 \pm 23	82 \pm 8.7
24 h	2.9 \pm 0.2	56 \pm 15	118 \pm 39

^aIA = iophenoxic acid.

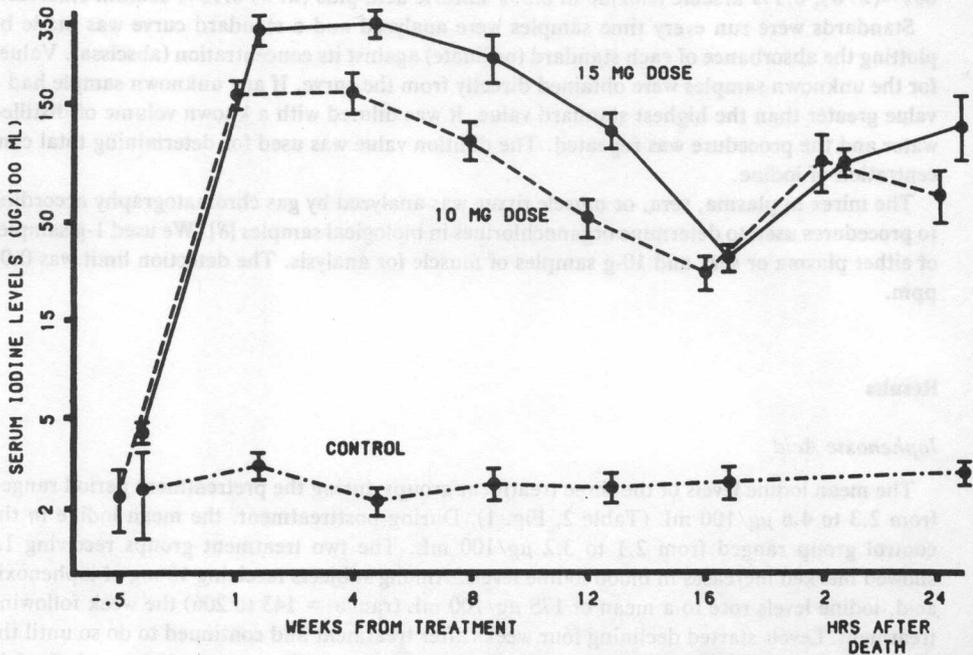


FIG. 1—Serum iodine levels (mean \pm SE) of coyotes fed baits containing single doses of 0, 10, or 15-mg iophenoxic acid. The control and 15-mg dose values have been purposely offset so that the standard errors could be displayed legibly.

treatment groups, iodine levels typically varied two to threefold among subjects, but all levels were substantially higher than controls.

Mirex

Mirex was not detected in any of the treatment groups in the pretreatment period and none was detected in the control group posttreatment (Table 3). Mirex was detected in all samples taken from mirex-treated coyotes throughout the posttreatment period (Fig. 2). Among the eight treated animals, mirex concentrations in sera or plasma averaged 0.34 ppm (range = 0.18 to 0.68) one week after treatment and slowly declined to 0.15 ppm (range = 0.02 to 0.41) by the end of 16 weeks. As with iophenoxic acid, mirex concentrations in sera samples taken 2 and 24 h after death were appreciably higher than concentrations in samples drawn just before death. Mirex concentrations in muscle tissues varied tenfold among subjects but in all cases were greater than levels detected in sera from the same animals.

Rhodamine B

Fluorescent bands on the guard hairs of the back were not noted prior to treatment nor the first week after treatment in any group. Subsequently, some but not all back guard hairs removed from the coyotes that had been fed 150 mg of rhodamine B revealed one unambiguous fluorescent band somewhere along the hair shaft. Fluorescent bands were noted on some guard hairs of all eight treated animals four weeks after treatment. At 8 and 12-weeks posttreatment, fluorescent bands were recognized on seven of the eight treated coyotes. (Data from Week 16 posttreatment were misplaced and unavailable for analysis.) The fluorescent band was created in the hair follicle and became progressively more distant from the follicle during the latter weeks of the study.

Examination of toenails, vibrissae, hairs between the toes, and tail guard hairs for fluorescence did not reveal distinct and dependable bands. In some cases obvious fluorescence was noted, while in others the band was absent or questionable and invariably obscured before Week 12. Fluorescent bands on hairs of the undercoat were never detected during macroexamination.

TABLE 3—Concentration of mirex (ppm \pm SE) in sera, plasma, and muscle of coyotes.

Time	Tissue	Treatment	
		Control (n = 4)	100 mg mirex (n = 8)
Pretreatment	sera	ND ^a	ND
Posttreatment			
1 week	plasma	ND	0.34 \pm 0.06
4 weeks	plasma	ND	0.21 \pm 0.04
8 weeks	sera	ND	0.20 \pm 0.07
12 weeks	sera	ND	0.17 \pm 0.04
16 weeks	sera	ND	0.15 \pm 0.05
Postdeath			
2 h	sera	ND	0.37 \pm 0.17
24 h	sera	ND	0.28 \pm 0.11
24 h	muscle	ND	1.74 \pm 0.76

^aND = Not detected; limit of detection = 0.02 ppm.

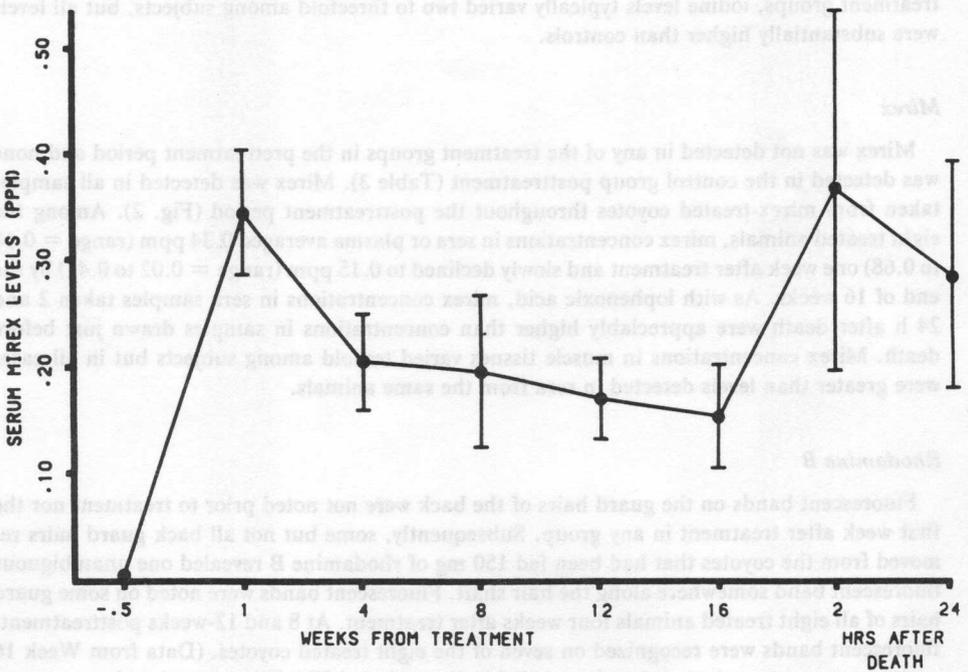


FIG. 2—Mirex concentrations (mean \pm SE) of coyotes fed baits containing single doses of 100-mg mirex.

Discussion

The first requirement we identified for a successful oral marking agent was met by all three agents tested because all were readily accepted and ingested by the captive coyotes.

The second and third requirements for physiological markers were met by IA and mirex since they produced distinct and unambiguous "marks" which persisted throughout the 16-week test. The 10 and 15-mg IA treatments could probably have been readily distinguished from controls for an additional four to eight weeks, but it is questionable whether such a discrimination would have been possible at 34 or 52 weeks as reported for red fox and domestic dogs, respectively [3]. Blood iodine concentrations we observed after eight weeks were greater than reported for coyotes [2] but probably reflect the higher doses of IA used in this study. Among coyotes ingesting the mirex marker, circulating levels of mirex declined by one third in the first month. The subsequent average rate of decrease in mirex concentrations in the sera was about 0.02 ppm per month. There seems little doubt that mirex could serve as an effective marker for a longer period than we tested, provided no environmental source of the agent is available (for example, areas where mirex may be used in broad applications to control fire ants).

Reasons for the elevation of iodine and mirex concentrations in samples obtained after death of the coyotes is unclear. We are not aware of similar observations by other investigators. Such amplification of the "marks" enhances the practicality of using these marking agents and reduces constraints upon the methods of animal recovery and sample collection.

Interpretations of the results obtained using rhodamine B as an oral marking agent are less certain. Most of the animals that ingested rhodamine B were readily identified twelve weeks later, and the "marks" probably persisted beyond the 16-week test period. Failure to identify one treated animal in each of the eight- and twelve-week examinations (different animals were

involved each time) possibly resulted because not all guard hairs carried the "mark" and perhaps not enough hairs were removed to provide adequate samples to detect the marks. Since the marks were created only during hair development, more information is needed regarding the patterns of hair growth in coyotes. The suitability of rhodamine B as a physiologic marking agent during seasons of the year when hair growth is reduced needs to be demonstrated.

The determination of iodine and mirex in plasma and sera is convenient and eliminates the need to sacrifice animals to acquire samples. These tissues are also convenient because relatively little effort is needed to prepare the samples prior to analysis. Blood iodine concentrations, however, are an indirect measure of IA; a direct assay would be preferable. Mirex can be detected in other tissues, and concentrations are frequently greater than those observed in sera or plasma, but additional sample preparation is needed prior to analysis. Although obtaining samples and assaying them for rhodamine B fluorescent bands involved simple procedures, uncertainties regarding the nature and placement of the bands need to be resolved before rhodamine B can be used as a reliable oral marking agent.

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