CHLORINATED BENZENES AS PHYSIOLOGICAL MARKERS FOR COYOTES

JOHN J. JOHNSTON,[1] U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, 3350 Eastbrook Drive, Fort Collins, CO 80525, USA
LAMAR A. WINDBERG, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, Utah State University, Logan, UT 84322, USA
CAROL A. FURCOLOW, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, 3350 Eastbrook Drive, Fort Collins, CO 80525, USA
RICHARD M. ENGEMAN, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, 1716 Heath Parkway, Fort Collins, CO 80525, USA
MARIANNA ROETTO, Box 357, Providence, UT 84332, USA

Abstract: We evaluated pentachlorobenzene (PeCB) and 1,2,4,5-tetrachlorobenzene (TeCB) as new, long-term physiological markers of coyotes (*Canis latrans*) for multipurpose research use. Captive coyotes were administered oral doses of 65, 130, 260, and 520 mg of PeCB in sesame oil, and samples of serum, wet (fresh) and dry feces, and adipose tissue were collected at 10 intervals over 168 days to measure concentrations of the marker. The PeCB concentrations in serum declined at an exponential rate over time. There were positive correlations between concentrations of PeCB in serum, wet and dry feces, and adipose tissue. Intramuscular injection of 65 mg of PeCB resulted in similar PeCB sample concentrations as the 65 mg oral dose. Oral administration of 50 mg of solid technical PeCB, along with 50 mg solid TeCB, resulted in lower sample concentrations than the 65-mg oral dose in oil ($P = 0.02$). Oral doses of 99 mg PeCB and 48 mg of TeCB in mineral oil produced relatively similar (1.8 times) PeCB concentrations in serum and feces for 28 days. Sample concentrations resulting from single and combined oral doses of PeCB (99 mg) and TeCB (48 mg) did not differ ($P = 0.85$). We detected no pathological effects on coyotes from the doses of PeCB and TeCB administered in this study as assessed by visual observations, measurement of body condition, and examination of serum biochemistry and hematological parameters. We believe PeCB offers considerable potential for multiple research applications as a long-term physiological marker for coyotes because it permits researchers to correlate PeCB levels with other activities such as bait ingestion or livestock predation. Marking properties of TeCB are similar to PeCB but may have restricted application because TeCB is of limited solubility in oil vehicles.

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Physiological markers have been used to estimate animal abundance (Meslow and Keith 1968, Pelton and Marcum 1977, Davison 1980) and the proportion of populations that ingest baits containing both the marker and substances such as toxicants, oral vaccines, or chemosterilants (Savarie et al. 1992, Linhart et al. 1996). Physiological markers have been used for quantitative studies of behavior (Crabtree et al. 1989) and patterns of predation (Windberg et al. 1997), when dosing with the marker accompanies certain behavior such as livestock predation (Connoly and Burns 1990). Key criteria for physiological markers are as follows: (1) readily delivered to target animals, (2) safe for target and nontarget animals, (3) accurately detectable, (4) persistent for a requisite period, and (5) available without excessive restrictions for use. Other desirable features are that markers be relatively inexpensive and sampled by nonintrusive methods.

The availability of some effective markers is hindered by restrictions related to toxicity and environmental risks, although use as physiological markers may not pose a hazard. For example, mirex (and other chlorinated hydrocarbon pesticides) is a useful long-term marker (Knowlton et al. 1988), but the product is unavailable because its registration was canceled by the U. S. Environmental Protection Agency. Several radioisotopes provide satisfactory physiological markers of variable duration (Pelton and Marcum 1977, Knowlton et al. 1989), but their use is restricted and may be negatively perceived by the public (Crabtree et al. 1989). Iophenoxic acid and tetracyclines are safe and effective long-term physiological markers pres-

[1] E-mail: a347jjohnston@attmail.com

ently available for wildlife investigations (Knowlton et al. 1988, Savarie et al. 1992, Linhart et al. 1997), but they have sampling limitations. Hence, additional new wildlife markers that offer multiple applications are needed to replace inadequate substances.

Some lipophilic chlorinated benzene compounds offer the same desirable persistence and accurate detectability by gas chromatography as chlorinated hydrocarbon pesticides and have lower risk of toxicity. Furthermore, evaluation of 2 chlorinated benzenes, PeCB and 1,2,3,4-tetrachlorobenzene, showed potential as oral physiological markers for coyotes (Kimball et al. 1996). Residues of pentachlorobenzene in sera, feces, and adipose and muscle tissue of coyotes were detectable for 120 days following a single 100-mg dose of the compound. However, doses of 100-mg 1,2,3,4-tetrachlorobenzene resulted in detectable residues for ≤30 days post-dosing.

To evaluate PeCB and TeCB for use as a physiological marker for wildlife studies and management, our objective was to determine the persistence per oral dose of PeCB in samples of serum, wet (fresh) and dry feces, and adipose tissue of coyotes. We also wished to evaluate if residue levels were affected by either the carrier used for delivery of the physiological markers or administration of the markers in a single versus multiple dose.

METHODS

Animal Procedures

We housed coyotes (2–12 yr old) in covered outdoor kennels (1.2 × 3.6 × 1.8 m) at the U.S. Department of Agriculture Predator Research Facility located 12 km south of Logan, Utah, USA. Animal use procedures were approved by the Institutional Animal Care and Use Committee of the Denver Wildlife Research Center. Coyotes received a daily ration (approx 300 g) of commercial dry dog food (Hill's Pet Nutrition, Topeka, Kansas, USA). For dosing and blood sampling, we immobilized coyotes with an intramuscular (IM) injection of 100 mg of ketamine hydrochloride (Aveco Company, Fort Dodge, Iowa, USA) and 1 mg of acepromazine maleate (Fermenta Animal Health, Kansas City, Missouri, USA). For surgical sampling of adipose tissue, we anesthetized coyotes with an intravenous dose of sodium thiopental at 4mg/kg (Abbott Laboratories, North Chicago, Illinois, USA). We determined mass of coyotes to the nearest 0.1 kg on a platform scale and used a steel tape to measure body length (tip of nose to base of tail) to the nearest 0.1 cm. For an index of subcutaneous adipose tissue (Whittemore 1984), we used a Harpenden skinfold calipers to measure skinfold thickness to the nearest 0.1 mm between the scapulae in the dorsolateral lumbar region.

Formulations

We obtained PeCB and TeCB from Aldrich Chemical, Milwaukee, Wisconsin, USA. For assessment of the relation between dose and sample concentrations, we formulated PeCB in sesame oil at 52 mg/mL. We measured the formulation into gelatin capsules (1.0–1.4 mL) for oral dosing. We used 2 procedures for comparison of delivery methods: (1) a portion of the above formulation was sterilized by autoclaving the IM injection formulation and was reanalyzed by gas chromatography to assure the concentration was unchanged, and (2) 50 mg of PeCB and 50 mg of solid technical TeCB were combined in 1 capsule. For comparison of single compared to combined doses of PeCB and TeCB, we formulated the compounds in light mineral oil (Aldrich Chemical) at concentrations of 49.5 mg/mL for PeCB and 18.6 mg/mL for TeCB.

Dosing Procedures

For assessment of the relation between dose and sample concentrations of PeCB, we randomly assigned 15 coyotes to 4 treatment groups of 3 animals, along with 3 untreated controls. Each group had ≥1 animal of each sex. We administered oral treatments of 65, 130, 260, and 520 mg PeCB to coyotes fasted approximately 10 hr. Individuals in the 3 lowest treatment groups received 1.3, 2.5, or 5.0 mL of formulation and were dosed by feeding the formulation in gelatin capsules during recovery from immobilization. Coyotes in the control and highest treatment groups received 10 mL of formulation each and were dosed via a gavage tube. Controls received 10 mL of sesame oil.

For comparison of delivery methods, we dosed 2 additional treatment groups of 3 coyotes each in conjunction with the above treatments. One group received an IM injection of 65 mg of PeCB in 1.25 mL of formulation. We used a syringe to inject the dose into the caudal thigh muscles at 2 sites on a rear leg, after shearing the hair and disinfecting the skin sur-

face. We orally dosed coyotes in the other group with a combination of 50 mg of PeCB and 50 mg of solid technical TeCB by feeding individuals a gelatin capsule during recovery from immobilization.

Because some field applications may require use of different markers, coyotes may potentially receive 2 chlorinated benzenes. Hence, we compared concentrations of PeCB and TeCB in animals treated with single doses of each compound to animals treated with a combination of both compounds. Fourteen additional coyotes were randomly assigned to 3 treatment groups of 4 animals, and 2 coyotes served as untreated controls. Each group comprised equal numbers of males and females. The first group was administered an oral dose of 99.0 mg of PeCB, the second group received 48.4 mg of TeCB, and the third group received 99.0 mg of PeCB and 48.4 mg of TeCB. We fed markers to coyotes in gelatin capsules containing 4 mL of formulation; controls received 4 mL of mineral oil.

Sampling Procedures

We determined PeCB and TeCB concentrations in both wet and dry feces. We manually homogenized composite samples, and 3 subsamples (0.5–1.0 g) were removed and frozen (−24°C) until analysis. We prepared dry fecal samples by placing 15–60 g of wet feces in a forced-air laboratory oven at 35–40°C for 6 days; mass of 3 subsamples was determined and samples preserved at room temperature. We determined moisture loss of feces from pre- and postdrying mass.

We extracted 20-mL blood samples from the cephalic vein of coyotes fasted approximately 10 hr. Blood serum was separated by centrifugation, frozen, and 3 subsamples (0.5–1.0 mL) were analyzed to determine the concentration of physiological markers. We excised samples of adipose tissue (1.5–2.5 g) from the deposit at the falciform-ligament from which we determined the mass of 3 subsamples (0.5–1.0 g); samples were frozen until analysis.

For assessment of the relation between dose and sample concentrations of PeCB, we collected serum and wet fecal samples from coyotes at 35-days pretreatment and 1, 3, 7, 14, 28, 56, 112, 140, and 168 days posttreatment. We prepared oven-dry fecal samples at 3, 28, 56, 112, 140, and 168 days posttreatment. We collected samples of adipose tissue at 30–40 days ($\bar{x}$ = 35 days) pretreatment and 111–118 days ($\bar{x}$ = 114 days) posttreatment. To compare PeCB in feces produced from diets of dog food compared to representative prey, we fed coyotes ≥600 g of rats (*Rattus norvegicus, Neotoma micropus*) at 168–169 days posttreatment. We collected coyote feces from the prey diet at 170 days posttreatment and prepared wet and dry fecal samples as described above.

For comparison of delivery methods, we collected samples of serum, wet feces, and adipose tissue at the same intervals listed above, excluding 140 and 168 days posttreatment. For comparison of single and combined doses of PeCB and TeCB, we collected samples of serum and wet feces at 7 days pretreatment and 1, 3, 7, 14, and 28 days posttreatment.

Sample Preparation and Analytical Chemistry

We extracted subsamples (0.5–1.0 mL) of serum, feces, and adipose tissue in triplicate with 1 mL of toluene and vortex mixing. We quantified concentrations of markers by capillary gas chromatography-electron capture detection (Johnston et al. 1997). We fortified triplicate quality-control samples for each sample type at concentrations approximating the expected results on each day of analysis. We used the mean quality-control recoveries to normalize the final concentrations. To ascertain the stability of physiological marker residues during the drying process, we corrected the dry fecal sample residue data for water loss.

Toxicity Data

We used 3 procedures to assess potential toxic effects related to treatment with PeCB and TeCB: (1) routine visual inspection of coyotes for abnormal symptoms; (2) measurement of body mass and subcutaneous adipose tissue at 28-day intervals; and (3) examination of complete blood profiles at 35 days pretreatment, and 1, 7, and 28 days posttreatment. Procedures for determining 20 serum biochemical and 16 hematological parameters were described by Kimball et al. (1996). We compared blood parameters (1) between pre- and posttreatment samples for treated coyotes, (2) between treated and control animals, and (3) with the normal ranges for domestic dogs (Duncan and Prasse 1986).

Data Analysis

We analyzed potential changes in concentrations of PeCB and TeCB in serum and feces stored frozen ≤60 days by linear regression analyses of residue results compared to time of storage; storage losses were indicated by a negative slope over sampling intervals. We analyzed effects of PeCB dose on sample concentrations in serum and wet and dry feces over time by 2-factor repeated-measures analysis of variance (ANOVA); differences among means were analyzed by Duncan's multiple-range test (DMRT). We used Pearson's correlations to quantify relations between sample concentrations in serum and wet feces, wet and dry feces, and serum and adipose tissue. To estimate the relation between dose and sample concentrations of PeCB, we modeled residue concentrations in the serum over time as an exponential decay equation for each concentration. We analyzed mean concentrations of PeCB in adipose tissue among treatments by 1-way ANOVA. We used a *t*-test to compare mean concentrations of PeCB in wet and dry feces produced from diets of dog food compared to rats. We used 3-factor repeated-measures ANOVA to analyze sample concentrations of markers in serum and feces resulting from (1) 3 methods of dosing, and (2) single and combined doses.

We compared mean body mass of male and female coyotes with a 2-sample *t*-test. We used linear regression to assess the relation between the dose of PeCB and TeCB per kilogram of body mass and sample concentrations in serum and feces between sexes. The percent decline in concentrations of PeCB and TeCB in serum from 1 to 28 days post-dosing was analyzed among sex and age classes by 1-way ANOVA. We used linear regression to analyze the relations between percent decline in marker concentrations in serum (1–28 days), body mass, and skinfold thickness at dosing. We used 1-way ANOVA to analyze mean body mass and skinfold thickness of coyotes sampled at 28-day intervals, and analyzed means for serum biochemical and hematological variables among treatment groups of coyotes and over sample intervals. Prior to ANOVA, we transformed hematocrit and the percent distribution of red blood cells according to Winer (1971) and confirmed normalcy of the transformed data (SAS Institute 1989).

RESULTS

Both PeCB and TeCB were more soluble in mineral oil than sesame oil; PeCB was about twice as soluble as TeCB in both oils. We used sesame oil as the vehicle for the IM dose in this study because it offered slightly greater solubility than other vegetable oils available in the pharmaceutical grade required for the IM injection. However, due to the relatively limited solubility of TeCB in sesame oil, it was not evaluated with PeCB, because we considered the volume of oil required to achieve the desired dose to be excessive. The concentration of the biomarkers in the dose formulations was about 90% of the maximum solubility at room temperature, and autoclaving had no effect on PeCB and TeCB concentrations.

For the physiological markers to be considered detectable in samples, we required marker concentration to exceed the chromatographic background by ≥2 times. This method limit of detection (MLOD) varied somewhat among types and dates of samples. The mean MLOD (ng PeCB of sample) was 6.2 in serum, 11.6 in wet feces, 40.6 in dry feces, and 7.4 in adipose tissue. The mean MLOD (ng TeCB/g of sample) was 6.7 in serum, 15.5 in wet feces, 25.5 in dry feces, and 12.0 in adispose tissue. Concentrations of PeCB and TeCB in all pretreatment samples were less than MLOD. Concentrations of PeCB and TeCB in serum and feces were unchanged by 60 days of frozen storage.

Sample Concentrations and Dose

Mean concentrations of PeCB in serum and wet and dry feces declined ($F_{3,8} = 15.44$, $P = 0.03$) for the 4 treatment groups from 1 to 168 days post-dosing (Fig. 1). The effect of doses of 65, 130, 260, and 520 mg of PeCB yielded differences ($P < 0.001$; DMRT) in concentrations in serum and wet feces. The PeCB concentrations in feces on day 1 varied greatly among individuals (mean CV = 103%).

When we excluded the 520-mg treatment group, the declines in concentration of PeCB in serum from day 1 to day 168 fit an exponential regression for each of 9 coyotes ($r^2 = 0.64$–0.97; $t_7 = 3.5$–15.2, $P \leq 0.01$). Data for the 520-mg treatment were not included in the modeling efforts, because the decline in concentrations for that group did not correspond well with the pattern of the others (Fig. 1). These animals were dosed via a different procedure, which

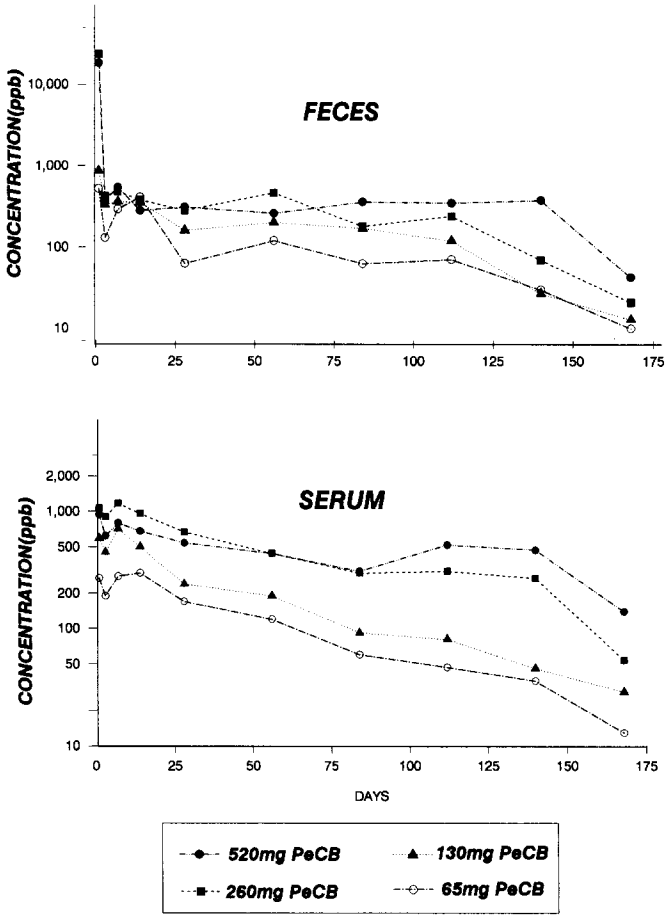

Fig. 1. Mean concentrations of PeCB in wet coyote feces (top) and coyote serum (bottom) from day 1 to day 168 for 4 doses (65, 130, 260, and 520 mg) delivered orally in sesame oil.

had limited potential for field application. Mean concentrations of PeCB in serum and wet feces for all doses administered in capsules were positively correlated (r = 0.86; t_{29} = 8.8, P < 0.001), as were the mean concentrations of PeCB in wet and dry feces (r = 0.80; t_{29} = 6.7, P < 0.001; Fig. 2). Excluding day 1, sample variability of PeCB concentrations generally was similar for serum (mean CV = 32%), wet feces (mean CV = 38%), and dry feces (mean CV = 39%).

For 17 coyotes at 114 days post-dosing, concentrations of PeCB in adipose tissue averaged 34 times more and were positively correlated with concentrations in serum (r = 0.81; t_{15} = 5.4, P < 0.001; Fig. 3). The mean PeCB concentration in adipose tissue was greater (P < 0.01; DMRT) for the 520-mg treatment ($\bar{x}$ = 20,200 ppb, SE = 5,570, n = 4) than the 260-mg ($\bar{x}$ = 6,580 ppb, SE = 2,730, n = 4), 130-mg ($\bar{x}$ = 2,750 ppb, SE = 1,100, n = 4), and 65-mg ($\bar{x}$ = 1,670 ppb, SE = 420, n = 4) treatments (P < 0.05; DMRT).

We compared concentrations of PeCB in wet and dry feces on day 168 for coyotes that received the commercial dog food diet for 6 months compared to the same individuals on day 170, which were fed rats for 2 days. We were unable to detect differences in the mean concentrations for either wet feces ($\bar{x}$ = 22 [dog food] vs. 33 [rats] ppb; t_5 = 1.53, P = 0.20) or dry feces ($\bar{x}$ = 33 vs. 38 ppb; t_8 = 1.36, P = 0.22).

Sample Concentrations and Method of Dosing

Our comparison of 3 methods for administration of PeCB to coyotes (oral in oil, oral as a

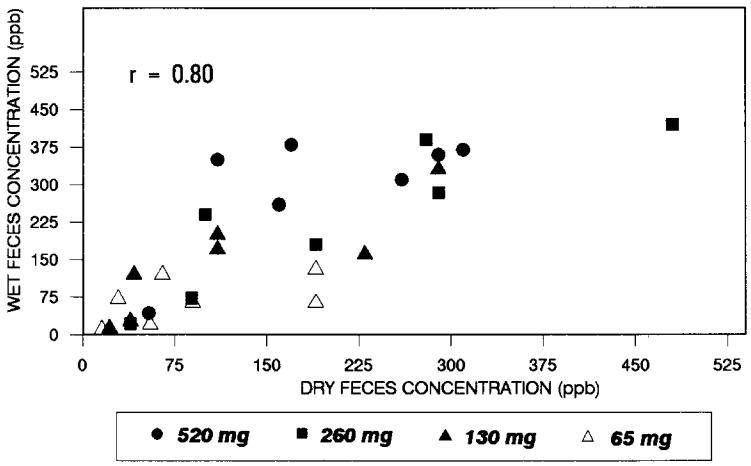

Fig. 2. Relation between mean concentrations of PeCB in wet and dry feces from day 3 to day 168 for coyotes dosed orally with 65–520 mg of marker in sesame oil.

solid, IM in oil) resulted in markedly different concentrations in feces 1 day later (Fig. 4). In coyotes dosed with the solid (50 mg), mean PeCB concentration was extremely high ($P < 0.05$; DMRT), whereas there was no PeCB (<MLOD) in feces of coyotes dosed IM (65 mg). The mean PeCB concentration was lower ($t_4 = 5.7$, $P < 0.01$) in serum on day 1 for coyotes dosed IM compared to those dosed orally via the oil vehicle. In coyotes dosed IM, there was a trend for PeCB in serum to increase progressively from day 1 to day 14 but to decline thereafter (Fig. 4). Mean PeCB concentrations in serum and feces of coyotes dosed orally did not differ from those injected IM from day 28 to day 112. Mean PeCB concentrations in adipose tissue at day 114 did not differ ($t_4 = 0.9$, $P = 0.41$) between coyotes dosed orally ($\bar{x}$ = 1,670 ppb, SE = 420) and IM ($\bar{x}$ = 3,200 ppb, SE = 1,580).

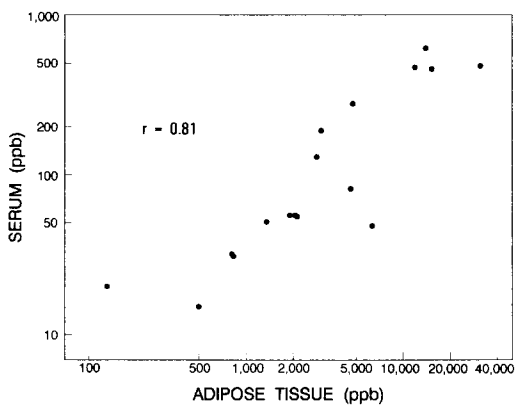

Fig. 3. Relation between concentrations of PeCB in serum and adipose tissue during 111–118 days posttreatment for 17 coyotes dosed with 50–520 mg of the marker in oil.

Mean PeCB in serum and feces of coyotes dosed with 50 mg of solid was less ($P < 0.01$) than those dosed with 65 mg of PeCB via oil and IM (Fig. 4). Mean TeCB in serum and feces in coyotes dosed with 50 mg of solid material followed a similar ($P > 0.50$) pattern as those dosed with 50 mg solid PeCB from day 1 to day 112 (Fig. 4). Mean TeCB ($\bar{x}$ = 180 ppb, SE = 87) and PeCB ($\bar{x}$ = 210 ppb, SE = 150) in adipose tissue at 114 days did not differ ($t_4 = 0.2$, $P > 0.50$) among coyotes treated with the solid doses.

Single Versus Combined Doses

Administration of single and combined doses of 99 mg of PeCB and 48 mg of TeCB in mineral oil resulted in relatively high concentrations of the markers in feces on day 1 (Fig. 5). The concentrations of both markers in coyotes that received single compared to combined doses were not different in either serum (PeCB: $F_{1,6} = 1.38$, $P = 0.28$; TeCB: $F_{1,6} = 0.38$, $P = 0.56$) or feces (PeCB: $F_{1,6} = 0.03$, $P = 0.87$; TeCB: $F_{1,6} = 0.31$, $P = 0.60$). Overall, the dose of PeCB yielded mean concentrations in serum and feces that averaged 1.8 times more than the dose of TeCB. This difference indicated that, per unit of material, the persistence of the 2 markers was similar for 28 days. A sample of adipose tissue from 1 coyote that died 196 days after dosing with 48 mg of TeCB had a concentration of 18 ppb TeCB.

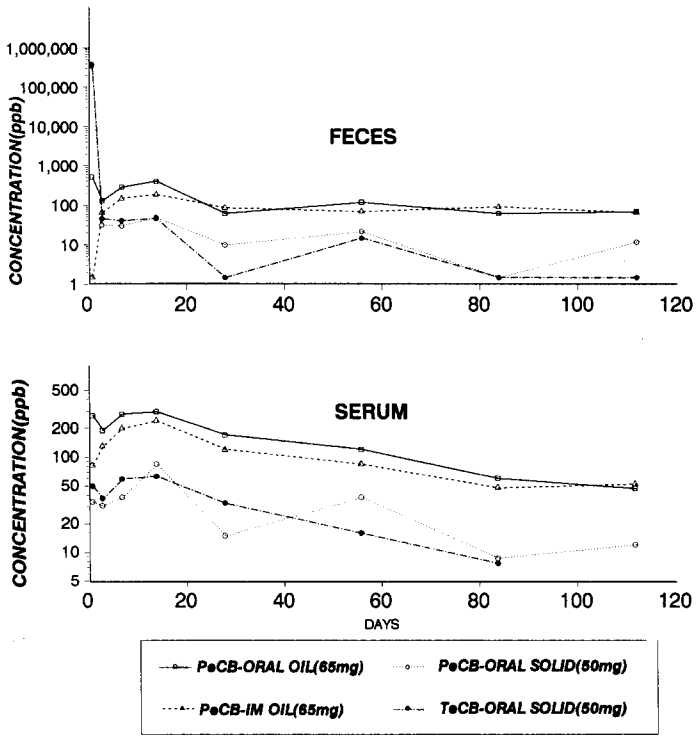

Fig. 4. Mean concentrations of PeCB in coyote serum and feces (wet) from day 1 to day 114 for doses delivered orally in sesame oil (65 mg), intramuscularly in sesame oil (65 mg), and orally as solid technical (50 mg), and TeCB delivered orally as solid technical (50 mg).

Sample Concentrations and Body Condition

Mean body mass of male coyotes ($\bar{x}$ = 12.5 kg, SE = 2.4) was greater (t_{33} = 4.4, $P < 0.001$) than females ($\bar{x}$ = 10.8 kg, SE = 3.0). Because the quantity of marker administered was constant for each treatment group, females generally received higher doses than males per kilogram of body mass. However, when we converted doses to milligrams of PeCB and TeCB per kilogram of body mass, we found no relation ($\bar{x}$ for r_2 = 0.19) between the dose of PeCB and TeCB and the resultant marker concentrations in serum and feces.

We detected no differences in the percent decline in concentrations between sexes ($F_{1,19}$ = 0.55, $P > 0.50$). Also, there was no detectable relation ($ts \leq 1.5$, $Ps \geq 0.16$) between percent decline in concentrations of either marker and coyote body mass (r_{11} = 0.28; $F_{6,14}$ = 7.44, n = 27) or skinfold thickness (r_{11} = 0.0; $F_{6,14}$ = 0.93, n = 27).

Assessment of Toxicity

All coyotes used as test subjects maintained good health throughout the study. However, most coyotes had relatively mild diarrhea at dosing on 16 January 1995. Coyotes dosed on 2 May 1995 had normal fecal passages.

Our visual examinations during post-dosing revealed no evidence of granulomas, abscesses, or other pathological changes at the injection sites of coyotes dosed IM with PeCB. There were no differences between pre-and post-dosing in the appearance of eyes, gums, teeth, or fur. Mean body mass of coyotes was unchanged ($F_{6,14}$ = 0.93, $P > 0.50$) among all treatment groups from time of dosing to day 168. Mean skinfold thickness of coyotes increased ($F_{6,14}$ = 7.44, $P < 0.001$, n = 21) from dosing ($\bar{x}$ = 2.3 cm, SE = 0.1) to day 112 ($\bar{x}$ = 4.4 cm, SE = 0.2). The majority of serum biochemistry and hematological variables were normal (Table 1).

DISCUSSION

An important consideration when evaluating dosages of physiological markers for wildlife is whether tests with captive animals provide data applicable to free-ranging animals. We believe our data are applicable to wild coyotes as daily

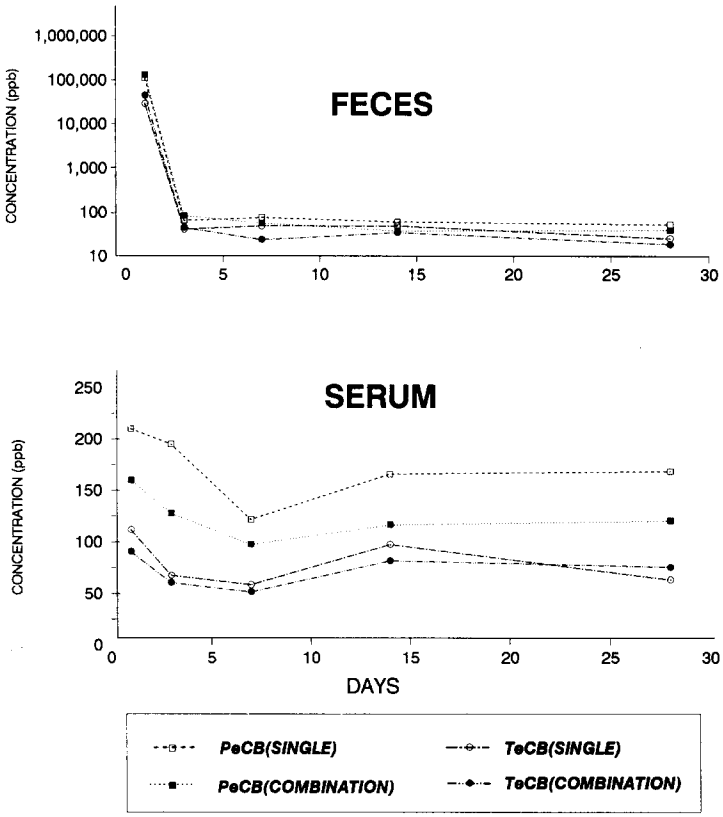

Fig. 5. Mean concentrations of PeCB and TeCB in coyote serum and feces (wet) from day 1 to day 28 delivered orally in mineral oil as single and combined doses of 99 mg of PeCB and 48 mg of TeCB.

energy expenditures of free-ranging mammals are only about 15% greater than captives (Nagy 1987), but the energetic cost of daily movements in wild mammals is a relatively small part (0.5–4.2%) of energy expenditure (Robbins 1993). Our captive coyotes were housed under ambient conditions and fed a diet designed to maintain them in body condition representative of wild coyotes. Hence, our data from captives are likely applicable to wild coyotes because adipose deposits of captives were assumed similar to wild coyotes (based on skin thickness), which should result in similar rates of absorption and metabolism of the physiological markers.

Pentachlorobenzene

Our study demonstrated potential for use of PeCB as a long-term physiological marker for coyotes. We found that resulting concentrations of PeCB in coyotes were approximately 4 times greater from dosing via sesame oil than with the solid technical material. However, the quantity of PeCB that can be delivered orally in oil is limited because of the finite solubility of PeCB in oil and because large volumes of vegetable oils increase gastrointestinal motility (Gad and Chengelis 1988). The volume of mineral oil that can be administered orally is even less, because it produces laxation (Hazardous Substances Databank 1994). Our data indicate vegetable oil to be the most practical vehicle for applications requiring oral dosing.

We evaluated dosing of coyotes by IM injection because that technique would be most practical for use with captured animals (Meslow and Keith 1968, Pelton and Marcum 1977, Crabtree et al. 1989). However, administration of markers by syringe injection raises safety issues such as irritation, pyrogenicity, blood compatibility, and sterility (Gad and Chengelis 1988). The IM route generally causes less pain and local irritation compared with subcutaneous injection, but potential complications can include infections and nerve damage (Gad and Chengelis 1988); we observed no pathologic changes from the IM dose of PeCB. Chemicals

Table 1. Mean serum chemistry and hematological variables for 3 untreated controls and 3 coyotes dosed with 520 mg of PeCB at predosing and 1, 7, and 28 days post-dosing.

Blood variables	Units	Control				520 mg of PeCB			
		Predose	Day 1	Day 7	Day 28	Predose	Day 1	Day 7	Day 28
Serum chemistry:									
Sodium	mEq/L	147	146	150	148	147	146	149	147
Potassium	mEq/L	4.5	4.4	4.6	4.4	5.3	4.9	5.1	5.0
Chloride	mEq/L	118	117	121	121	119	117	121	121
Carbon dioxide	mEq/L	18	15*[a]	18	15*	17	16*	18	17
Glucose	mg/dL	98	89	97	94	117	91	97	102
Blood urea nitrogen	mg/dL	23	18	24	20	24	25	28	25
Creatinine phosphokinase	mg/dL	1.0	1.0	1.2	1.1	0.9	1.1	1.1	1.1
Calcium	mg/dL	9.2	9.1	9.6	9.5	9.2	9.0	9.4	9.2
Magnesium	mg/dL	2.1	1.9	2.0	1.9	2.1	2.1	2.2	2.1
Phosphorus	mg/dL	3.6	3.6	5.4	4.6	4.7	4.2	5.7	5.2
Total protein	g/dL	6.5	6.5	6.5	6.5	5.9	6.2	6.2	6.2
Albumin	g/dL	3.6	3.6	3.6	3.7	3.3	3.6	3.6	3.6
Uric acid	mg/dL	0.3	0.3	0.2	0.4	0.2	0.2	0.2	0.2
Cholesterol	mg/dL	162	161	162	162	227	244	234	244
Triglycerides	mg/dL	42	34	49	40	88	62	62	46
Total bilirubin	mg/dL	0.2	0.2	0.3	0.2	0.3	0.2	0.3	0.2
Alkaline phosphatase	IU/L	26	29	29	32	30	38	37	32
LDH[b]	IU/L	157	233	166	114	174	202	179	171
ALT[b]	IU/L	41	63*	54*	53*	33	45	46	39
AST[b]	IU/L	38	94*	38	38	24	91*	33	31
Hematology:									
Total white blood cells	$n/\mu L$	8,033	7,533	7,400	6,633*	7,500	7,433	6,267*	6,067*
Total red blood cells	$n \times 10^6/\mu L$	6.8	6.7	6.6	6.8	6.2	6.7	6.6	6.7
Hemoglobin	g/dL	16.1	15.6	15.3	16.2	14.9	15.5	15.3	16.1
Hematocrit	%	47.0	46.2	44.8	46.7	42.8	45.5	44.4	45.2
$\bar{x}$ corpuscular volume	fL	68.7	68.9	68.3	68.5	68.6	68.0	68.2	67.6
$\bar{x}$ corpuscular hemoglobin	pg	23.9	23.2	23.4	23.9	24.0	23.2	23.2	24.2
MCH concentration	g/dL	34.2	33.7	34.2	34.8	34.9	34.1	34.0	35.7
RBC distribution width	%	14.6	13.4	13.3	13.3	14.1	14.0	13.8	13.2
Platelet estimate	$n \times 10^3 \mu L$	425	391	409	339	655	438	456	430
$\bar{x}$ platelet volume	fL	7.9	8.2	8.1	8.5	8.1	8.1	8.0	8.4
Band neutrophils	$n/\mu L$	329*	731*	531*	108	390*	982*	177	99
Segmented neutrophils	$n/\mu L$	7,389	5,317	4,834	5,092	5,291	5,154	4,067	4,809
Lymphocytes	$n/\mu L$	1,214	1,234	1,828	1,117	1,281	867*	1,602	843*
Monocytes	$n/\mu L$	186	94	101	68	260	304	117	95
Eosinophils	$n/\mu L$	233	157	105	248	579	126	303	220
Basophils	$n/\mu L$	0	0	0	0	0	0	0	0

[a] Values with an asterisk are outside of normal range for dogs.
[b] LDH = lactic dehydrogenase; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

in an oil vehicle form a depot in muscle tissue from which absorption proceeds slowly (Gad and Chengelis 1988). Hence, when compared to residues in orally dosed coyotes, we expected that a greater proportion of the IM PeCB dose might bioaccumulate in coyotes. We observed a progressive increase in PeCB concentrations in serum over the initial 14 days among coyotes dosed IM. However, the subsequent concentrations of PeCB did not differ between the 65-mg IM and oral (oil) treatments. For medium to long-term studies (>14 days), IM injections appear to offer no advantage over oral dosing.

Because concentrations of PeCB were markedly greater in adipose tissue than sera or feces, adipose samples offer considerably longer detectability of markers, but adispose tissue can only be obtained by postmortem or surgical sampling. Collection of sera and feces are more practical for field studies. Mean PeCB levels in sera and feces were slightly above the MLOD at the 168-day sampling interval. Extrapolation of the higher dose curves indicates that, for higher doses, PeCB residues would be detectable for at least 200 days post-dosing. The presence of PeCB in coyote sera offers broad field application because blood can be readily sampled from live animals (Savarie et al. 1992, Linhart et al. 1996).

The PeCB is also well suited for studies requiring feces sampling for marker detection because concentrations of PeCB in coyote feces correlated well with concentrations in sera. However, sampling of feces for physiological markers has only been used for radioisotopes (Pelton and Marcum 1977, Davison 1980) and physical markers (Savarie et al. 1992). Field samples of feces can have highly variable moisture content. Nevertheless, concentrations of PeCB can be quantified in field samples without correction for moisture differences because we found a strong correlation between wet and dry feces.

Coyote diets of small mammals or dog food resulted in similar concentrations of PeCB in feces, indicating that differences in diet do not appreciably alter concentrations of the marker. Importantly, we also noted no change in concentrations of PeCB or TeCB in serum or wet feces after frozen storage of samples for 60 days.

The use of gas chromatography to quantify PeCB concentrations in all types of samples can be useful in estimating the total quantity of marker ingested by free-ranging animals, which is required in certain field studies where wildlife coingest markers with other compounds such as rabies vaccines (Saunders et al. 1993, Larson et al., 1981). However, our estimates of concentrations of PeCB were subject to considerable variability, probably because absorption of test substances varies substantially among individual animals (Gad and Chengelis 1988). Some of the variability of the sample concentrations in sera possibly was related to differences in lipid profiles among individuals. Estimates for fecal samples had greatest variability, which was probably related to nonhomogeneous physical characteristics of the matrix and variable moisture content in wet feces. Also, chromatographic interferences were much greater for fecal samples than for sera and adipose tissue. The occurrence of a metabolite (2,3,4,5-tetrachlorophenol) of PeCB and TeCB in feces at greater concentrations than the marker may offer increased analytical sensitivity, and hence enhanced detectability (Johnston et al. 1997).

Tetrachlorobenzene

Our evaluation of TeCB as a physiological marker for coyotes was confounded by its limited solubility in oil vehicles. However, our data indicate that the marking properties of TeCB are similar to PeCB. The PeCB yielded nearly identical persistence as PeCB when administered orally in mineral oil and as a solid. There was greater chromatographic interference at the retention time for TeCB than PeCB, which slightly increased its MLOD.

We suggest that TeCB may potentially serve as a useful physiological marker and can be used in combination with PeCB to achieve similar sample concentrations when administered in a single dose. However, its low solubility limits its operational dose, and hence substantially reduces the period of detectability. Additional evaluation of TeCB as a marker for specific applications may be necessary.

Toxicity

The acute toxicity of PeCB and TeCB is relatively mild, as the oral LD_{50} concentrations for rats were 1,125 mg/kg for PeCB and 1,470 mg/kg for TeCB (Hazardous Substances Databank 1992*b*,*c*). In comparison, the highest single dose administered to coyotes in this study was approximately 45 mg/kg of PeCB, which we estimated to exceed the maximum dose for po-

tential field studies. We noted no evidence of acute toxicity in coyotes based on the lack of external symptoms and examination of serum biochemical and hematological variables 1 day after dosing with 6 treatments of PeCB and 1 treatment of TeCB.

The potential for subacute toxicity from ingestion of chlorinated benzenes poses the greatest risk to animals (Ariyoshi et al. 1975*a,b*; Chu et al. 1983; Hazardous Substances Databank 1992*a,b,c,*). Although our study involved only single doses of PeCB and TeCB, their absorption in adipose tissue and subsequent slow metabolism from the body still posed a potential subacute risk to coyotes. However, after examination of several variables, including hepatic function, we observed no evidence of toxicity at 7 and 28 days post-dosing.

MANAGEMENT IMPLICATIONS

We believe PeCB offers considerable potential as an effective, safe, long-term physiological marker for coyotes. This marker can be administered both orally and intramuscularly. Potential research applications for oral delivery in an oil vehicle include use as a marker in Coyote Lure Operative Devices (Marsh et al. 1982), in physiological marking collars (Windberg et al. 1997), in tallow baits (Knowlton et al. 1988), and in sachets of oral vaccine (Linhart et al. 1997). The solid material may have application as a marker by integration in solid bait such as polymer fish-meal baits (Linhart et al. 1997). The IM injection of the marker is a practical mode of dosing captured animals (Meslow and Keith 1968, Davison 1980, Crabtree et al. 1989). Detectability of the marker in serum, feces, and adipose tissue provides several sampling options for achieving research objectives.

The combination of oral dosing and monitoring of ingestion via analysis of feces is well suited to large-scale field studies and wildlife management operations such as the ingestion of rabies or contraceptive vaccines by target wildlife populations. The analytical instrumentation required is readily available in most modern analytical chemistry laboratories.

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