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Evaluation of Some Radioisotopes as Marking Agents for Monitoring Bait Consumption

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ABSTRACT: Minute quantities (1 to 20 μCi) of six gamma-emitting radioisotopes, manganese-54, zinc-65, selenium-75, iodine-125, iodine-131, and cesium-134, incorporated into nontoxic baits were found suitable as marking agents to detect bait consumption by coyotes (*Canis latrans*). The small quantity of isotope required minimizes detection of the marker by coyotes and potentially provides a bias-free procedure for simultaneously comparing the relative efficiency of several bait delivery techniques. Presence and identity of specific markers were determined with a multichannel analyzer on the basis of the energy level of emitted radiation. Differences in relative distribution of these isotopes among body tissues were noted. Other potential applications and constraints are discussed.

KEY WORDS: baiting, *Canis latrans*, coyote, oral markers, radioisotopes. Mn-54, Zn-65, Se-75, I-125, I-131, Cs-134

Studies related to baiting wild animals commonly involve incorporating a variety of agents into bait materials as a means of identifying the animals that ingest the bait. Such marking agents have varied from inert substances that simply adhere to or pass through the alimentary tract and are deposited in the feces [1,2] to materials that are absorbed by the body and accumulate in particular tissues and are recognizable because they "stain" [3-6] or chemically alter tissues [7-9] in ways that can be detected.

Although radioisotopes have been used in various ways to mark or identify animals [10-12] or their excretory [13-17] or reproductive [18,19] products, they have seldom been mentioned as marking agents to detect oral ingestion. Since most radioisotopes are recognizable by unique energies of emitted radiation, the number of potential markers is large compared to other types of oral physiological marking materials.

Three conditions must be met for oral marking agents to be useful in baiting studies, including (1) not deterring animals from ingesting the bait and (2) producing "marks" that

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are readily and individually detectable and distinguishable with (3) "marks" persisting for the required period of time [9]. Additionally, to avoid bias among baits, the marking agent should not be detectable by the target animal. With radioisotopes, concern about the first requirement is minimized because very minute quantities of isotopes are usually adequate to produce effective marks. The relative energies of radiation emitted from individual isotopes provide unique "signatures" which meet the second requirement if there is sufficient activity to raise radiation counts above background radiation and the active material has an adequate physical half-life and remains in the body long enough to meet the third requirement. Detectability is influenced by the length of assay (counting) time.

Preliminary evaluations of ten isotopes, sodium-22 (Na-22), manganese-54 (Mn-54), cobalt-57 (Co-57), zinc-65 (Zn-65), selenium-75 (Se-75), strontium-85 (Sr-85), yttrium-88 (Y-88), iodine-125 (I-125), iodine-131 (I-131), and cesium-134 (Cs-134), indicated four (Na-22, Co-57, Sr-85, and Y-88) were markedly less promising because they did not appear to produce lasting marks or were expensive or difficult to obtain. Herein we report subsequent evaluations of the other six isotopes.

Our specific objective was to assess the utility of Mn-54, Zn-65, Se-75, I-125, I-131, and Cs-134 as oral marking agents for use in coyote baiting studies by: (1) determining how well small quantities of the materials could be detected in the animals, (2) determining the tissues in which the strongest and most persistent marks occur, and (3) assessing the relative retention of marks over a 20-day period.

Methods

Subjects

Four adult coyotes (two males and two females) reared in the USDA Predator Research Facility at Millville, Utah, were transported to holding facilities of the Arid Land Ecological Reserve near Richland, Washington, where they were housed as pairs in cages 4 by 5 by 2.5 m. They were fed 1500 g per pen daily of a food ration prepared commercially for fur-farming operations and had water ad libitum.

Procedures

A series of 7.5-g baits made from beef tallow were used to facilitate ingestion of the desired amount of radioactive material. Baits were molded into a cone shape from melted tallow and a 1-cm³ cavity created in one end. A measured amount of radioactive material (dissolved in a very dilute acid) was placed in the cavity with a micropipette and the liquid allowed to evaporate overnight. The cavity was then filled with melted tallow and allowed to harden. Two experimental treatments were used with both coyotes in each cage receiving the same treatment. One treatment consisted of force-feeding tallow baits containing 10 μ Ci of Zn-65, 20 μ Ci of Mn-54, and 2.5 μ Ci of Cs-134 to both coyotes in one pen. Coyotes in the other pen each received baits containing 10 μ Ci of Se-75, 1 μ Ci of I-125, and 2 μ Ci of I-131. The dose of each isotope was determined empirically on the basis of published data on the physical half-life and gut-to-blood absorption rates (Table 1) [20,21].

Immediately before treatment, and on Days 2, 5, 10, 15, and 20 post-treatment, each coyote was anesthetized with an intramuscular injection of 100 mg of ketamine hydrochloride to facilitate transport to a laboratory and assay analysis. A 7-mL sample of blood was drawn into an evacuated collection tube and 100-s gamma radiation counts taken of the abdomen and throat regions with a multichannel pulse-height analyzer (Nuclear Data ND-60) attached to a germanium-lithium detector (Princeton Gamma-Tech 2) that was

TABLE 1—Comparison of physical and biological characteristics of isotopes^a evaluated in this study.

Isotope	Half-Life, Days			Gut-to-Blood Absorption Ratio	Preferred Tissue for Assay
	Physical	Biologic	Effective ^b		
Mn-54	300	25	23	0.1	liver
Zn-65	245	1959	218	0.1	muscle
Se-75	127	24	20	0.9	liver
I-125	60	138	42	1.0	thyroid
I-131	8	138	7.6	1.0	thyroid
Cs-134	840	140	120	1.0	muscle

^a Values presented from Refs 20 and 21.

^b Effective half-life incorporates physical half-life and biologic retention time.

cooled with liquid nitrogen. In the case of the abdomen, the animal was laid on its side and the detector placed slightly forward of the pelvis and directed dorsally and anteriorly toward the region of the kidneys and liver. Throat counts were made with the detector ventral to the neck and directed 1 to 2 in. (2.5 to 5 cm) below the larynx (region of the thyroid). This analyzer provided a printed output of the number of counts registered in each of 1024 channels representing an energy spectrum of 0.0 to 2.05 MeV. Since radiation energy from I-125 is too low on the energy spectrum to trigger the germanium-lithium detector, the thyroid region of the throat was also assayed with a single-channel pulse-height analyzer (Ludlum Model 2200) attached to a low-energy scintillator (Ludlum Model 44-3 thin-window sodium iodide detector) with a threshold setting of 275 and upper discriminator setting of 300, based on calibrations determined from a standard I-125 source. Counts were registered directly on, and recorded from, a light-emitting diode (LED) display on the device.

Subsequent to blood sampling on Day 20, the animals were euthanized with an intravenous injection of T-61 Euthanasia Solution (American-Hoecht, Inc.) and the thyroids and 5- to 8-g samples of skeletal muscle (hip region), liver, and kidney were removed, placed in individual plastic tubes, and stored under refrigeration. Logistical problems with the multichannel analyzer did not permit isotope assays of blood and tissue samples until 39 days after the tissues were collected. We compensated for this delay by using an assay time of 4 min each for the tissues. During assay, the tissue samples were left in the storage tubes and placed in a standard position 1 in. (2.5 cm) in front of the detector. Lead bricks partially shielded the detector from ambient radiation. Results were printed as described above.

Assays for Isotopes

Tallow baits containing the individual treatment doses of Mn-54, Zn-65, Se-75, I-131, and Cs-134 served as "standards" and were assayed with the multichannel analyzer to provide a sample of the unique pattern of radiation energies associated with each isotope (Fig. 1). By design, each of the isotopes tested had at least one diagnostic photopeak, or "spike," in the spectrum of radiated energies that was not ambiguous with the other isotopes. In addition to the diagnostic "spike" in radiated energies, the isotopes also produced a general increase in radiation as a result of scatter (Compton effect), especially in lower portions of the energy spectrum.

Since we were primarily interested in a qualitative assessment (presence/absence) we routinely checked the calibration of the analyzer with known sources (standards) and then

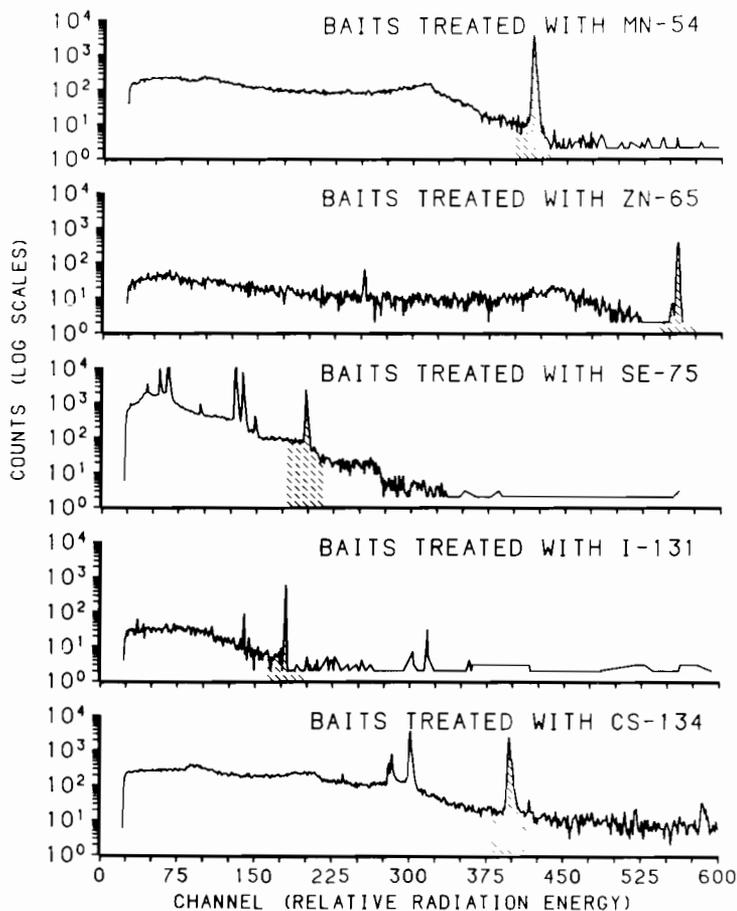


FIG. 1—Unique patterns of radiation energies for five of the six isotopes (incorporated in tallow baits) evaluated in this study based on total counts for 100 s with a germanium-lithium detector attached to a multichannel analyzer (measurements truncated at Channel 600 (1.20 MeV). Hatching indicates the diagnostic energy spikes used in this study.

used the numerical count recorded at our diagnostic energy channels for each isotope as a measure of the *peak* value. To assess the relative height of the peak with regard to the *adjacent channels*, we used the maximum and minimum radiation counts among ten channels above and below the peak energies, insuring that the adjacent channels did not include “shoulders” of the diagnostic spike (Fig. 2). Results were graphed on log-linear scales to provide sensitivity at low counts as well as a measure of the peak values.

Results

Location of Isotopes in Tissues

Radiation assays of blood, kidney, liver, muscle, and thyroid tissue collected 20 days after treatment revealed that, as expected, each isotope had stronger affinities for some tissues than others (Fig. 3). Kidney and liver tissue were excellent locations to detect Se-

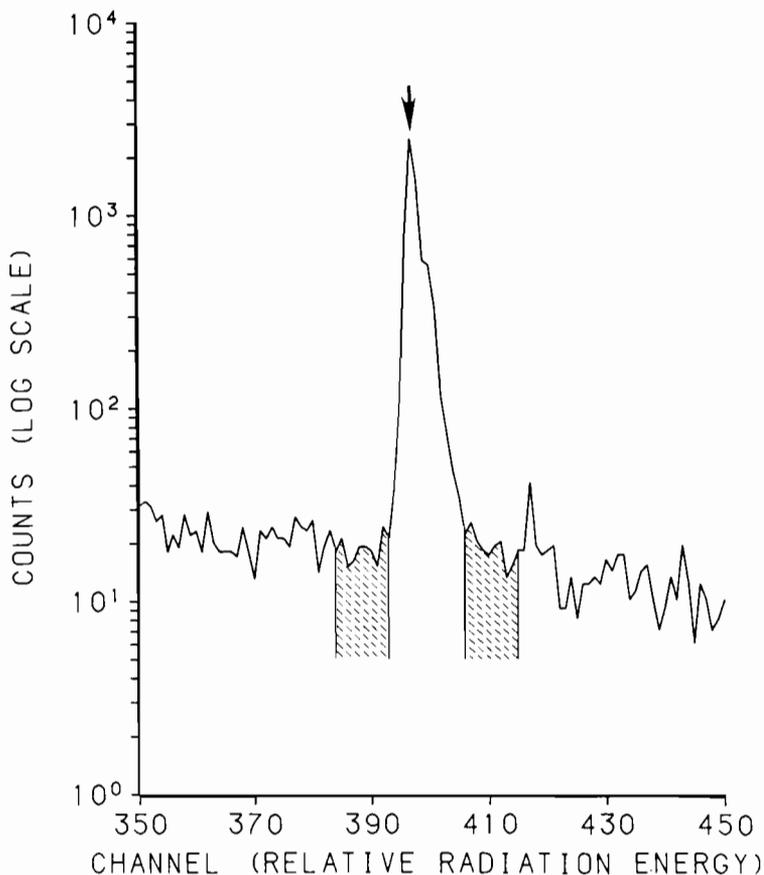


FIG. 2—Illustration of measurement locations for the diagnostic "spike" (arrow) and "adjacent channels" (hatched) from radiation energies of Cs-134.

75, Mn-54, and Cs-134, although the latter was most readily detected in muscle. Radiation levels from Zn-65 were highest in liver and muscle tissue. Both I-125 and I-131 were easily detected but after Day 5 were detectable only in the thyroid.

In general, blood collected 20 days after treatment was not effectively marked by any of the isotopes and only I-125 and I-131 produced satisfactory marks in the thyroids.

Durations of Marks

The marks produced by Mn-54, Zn-65, Se-75, and Cs-134 were easily recognized throughout the 20-day post-treatment period in both abdominal and throat assays of the live animals (Fig. 4). I-131 was also readily detected with the germanium-lithium detector, but only in assays in the region of the thyroid. In each case, peak radiation counts within the diagnostic energy channels were 10 to 100 times higher than the radiation counts in the energy channels adjacent to the peaks.

Assays for I-125 with the thin-window, NaI detector showed that pretreatment counts of the throats of the coyotes were low, but nearly double that of background (Table 2).

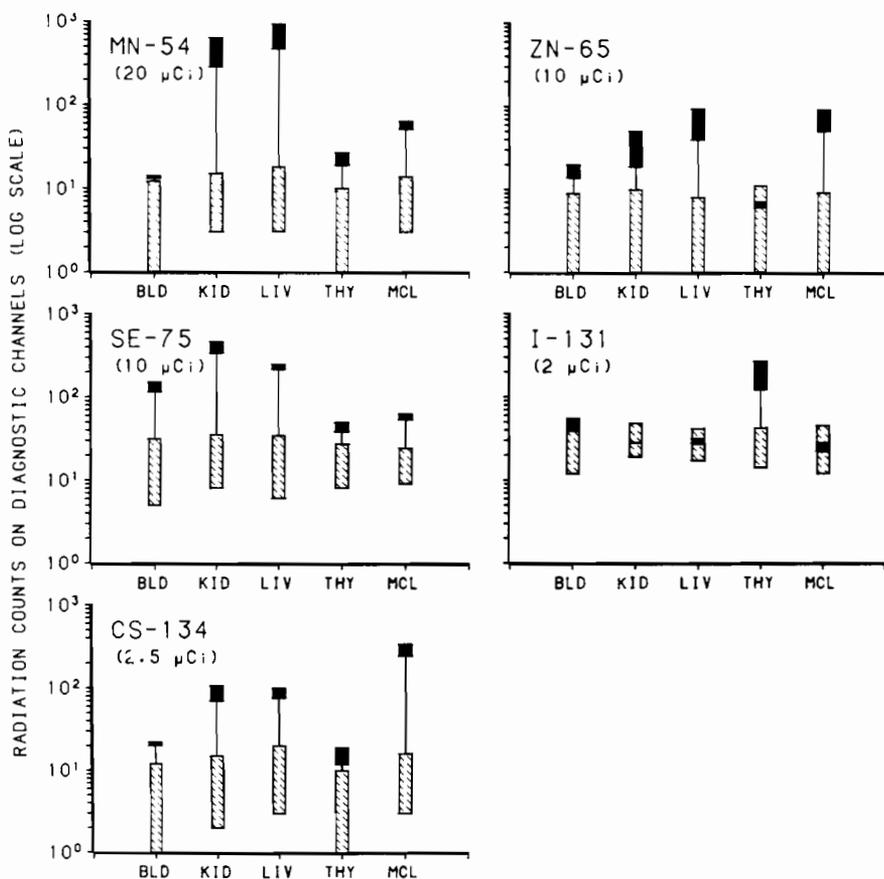


FIG. 3.—Ranges of 240-s radiation counts among tissues (BLD = blood, KID = kidney, LIV = liver, THY = thyroid, and MCL = muscle) from two coyotes. Differences between readings at diagnostic (solid) and adjacent channels (hatched) are indicated by the connecting lines.

Throughout the post-treatment period, throat counts of the 2 coyotes not treated with I-125 were 7 to 9 times greater than background probably as a result of radiation scatter associated with their treatment with other isotopes. Throat counts of the I-125-treated coyotes were 10 to 15 times higher than animals not treated with I-125, whereas counts on the excised thyroids from these animals were 130 to 150 times higher (Table 2).

Assays made 20 days after treatment revealed that counts for I-125, Mn-54, and Zn-65 ranged mostly from 70 to 100% of that detected on Day 5 (Table 3). Counts for cesium-134 exceeded 50% of that recorded on Day 5 while those for Se-75 and I-131 were generally below 50% (Table 3).

Discussion

Our goal was to assess qualitatively the practicality of using Cs-134, I-125, I-131, Mn-54, Se-75, and Zn-65 as oral marking agents that would be effective for ≥ 20 days in coyotes. All 6 isotopes ingested at the doses indicated exceeded the prescribed requirements.

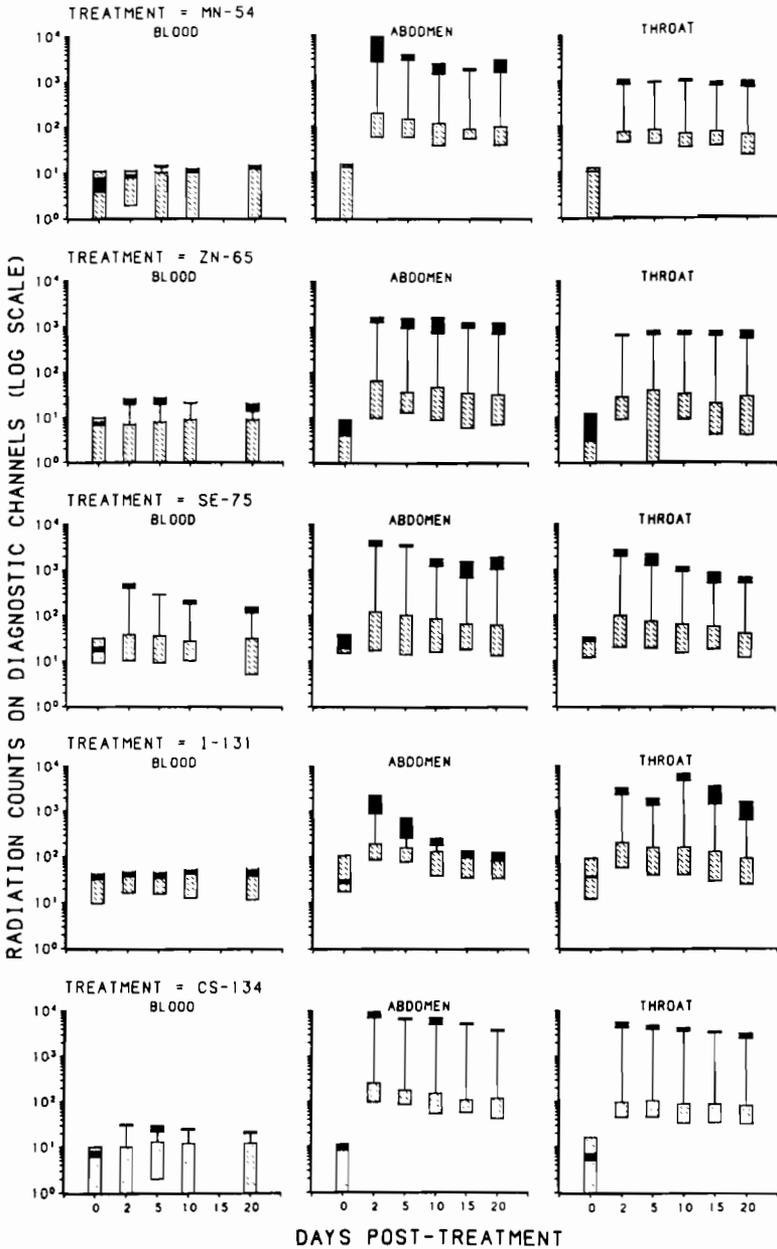


FIG. 4—Ranges of total radiation counts at the diagnostic (solid) and adjacent channels (hatched) in two live coyotes for each of five isotopes before and during the post-treatment period. Differences between readings at diagnostic (solid) and adjacent channels (hatched) are indicated by the connecting lines. The 100-s assays were made with a germanium-lithium detector attached to a multichannel analyzer.

TABLE 2—Comparison of 100-s total radiation counts of the thyroid region of control and I-125-treated coyotes made with a thin crystal sodium iodide detector.

Day	Treated Animals		Untreated Animals		Background Counts ^a
	3191	3245	2569	3205	
0	88	97	54
2	978	1 052	451	375	...
5	6 269	4 934	276	232	25
10	6 321	3 974	246	208	28
15	2 328	3 469	225	222	31
20	4 529	6 658	188	184	27
20 ^b	13 726	15 862	102	75	...

^a Determined immediately before, or after, subject counts.

^b Excised thyroid glands.

TABLE 3—Radiation count (100 s) at diagnostic peak energies for six isotopes on Day 20, expressed as a percent of the comparable peak count on Day 5.

Isotope	Survey Location						\bar{x}
	Abdomen			Throat			
	Animal		\bar{x}	Animal		\bar{x}	
A	B	A		B			
Mn-54	78	55	67	100	72	86	
Zn-65	78	73	76	100	82	91	
Se-75	57	29	43	31	41	36	
I-125	72	135	104	
I-131	31	17	24	46	80	63	
Cs-134	56	56	56	78	50	64	

Very strong, unambiguous marks associated with the radiation "signature" of each were readily discerned, even in the presence of the other isotopes. Substantially smaller doses would also have been readily detected over the prescribed interval.

The observed differential distribution of these elements within the body agrees with previously published information (for example, Refs 20 and 21) and underscores the need to understand anatomical and physiological fates of specified elements and compounds. Blood was generally a poor tissue to assay for the six isotopes we tested because of low concentrations of the markers. The thyroids are likewise poor assay locations except for the isotopes of iodine, in which case they are the essential tissue to assay. Liver, kidney, and muscle were all suitable to detect isotopes of Mn, Zn, Se, and Cs, with the preferred tissue varying slightly among the elements. If direct access to the detector with the animal is practical, all three tissues can be assayed simultaneously in an intact animal by placing the detector close to the abdomen and directing it toward the region of the liver and kidneys. More precise assessments are possible if the appropriate tissues are excised and submitted for laboratory analyses where more sensitive techniques and better shielding from background radiation can be achieved. This also translates into smaller dose requirements for creating a detectable mark.

In many cases the utility of a marking agent is related to the effective duration of the mark. In the case of isotopes, persistence of an identifying mark is a function of (1) the physical half-life of the isotope, (2) the biological half-life of the element in the animal, (3) the effective dose of the isotope administered, (4) the length of the assay (count) time, and (5) the inherent efficiency of the detector. Our primary interest involved recognition of the specific radioisotope mark rather than a quantitative measure of the radioactivity present. Our use of external detectors on live animals and the attendant difficulties of maintaining precise geometry between the detector and the various body organs decreased the precision of our quantitative assessments among assays. Thus, our comments regarding longevity of these isotopic marks are constrained by the procedures by which they were obtained.

The physical half-life is an inherent characteristic of individual isotopes and not subject to manipulation. Similarly, manipulation of biologic half-life in any given species is limited primarily to the molecular form in which the isotope is administered and the fate of such molecules in the body. On the other hand, the dose administered, tissues assayed, type and sensitivity of the detector(s), and the duration of the assay (counting time) are usually amenable to manipulation to meet the needs of specific studies.

Selection of isotopes for individual studies must include several considerations. The relatively long physical half-life of Mn-54 and Zn-65 are offset by low gut-to-blood absorption rates (Table 3). The other 4 isotopes evaluated all have high gut-to-blood absorption, but I-131 has a short (7.6-day) effective half-life, and I-125 requires a special detector because of its low energy emissions. Dose adjustments can partially compensate for a low effective half-life, but treatment with relatively low amounts of radioactivity is usually preferred. Our results suggest that the doses used here produced marks in coyotes which could have been recognized several months later, or that considerably lower doses would have been adequate for the 20-day test period.

Advantages of Isotopes as Oral Marks

There are a variety of radioisotopes that may be suitable as oral marking agents. Our original screening included ten, of which we subsequently tested six. Finding more is a matter of matching test requirements with the physical characteristics of the isotopes (half-life, types and energies of emitted radiation, and so forth), the biological attributes of the chemical form in which the isotope is available [20,21], the types of detection equipment available, and potential interference from naturally occurring sources of radiation. Multiple isotope markers could be important in the design and execution of many studies.

Analyses for radiotracers can be fast and relatively simple, and permit simultaneous assays for multiple marks if a multichannel analyzer can be used. Normally, sample preparation time is minimal and sacrifice of the subject is not necessary. The extremely small quantity of material needed to create the marks virtually eliminates concern about the marker itself influencing ingestion.

Constraints on Use of Radioisotopes as Marking Agents

Among the disadvantages associated with using isotope markers are stringent licensing requirements. Federal (Nuclear Regulatory Commission) or state (usually through the state Departments of Health) licensing and approval of individual projects is mandatory, and strict procedures and accountability are enforced regarding use, handling, and disposal of radioactive materials. Requirements for license approval and reporting need to be taken into account in the planning phases of studies.

Use of radioisotopes also requires significant commitments to "clean" technique and

protocol for the safety of the investigators and the integrity of the studies. Careful monitoring of personnel for contamination is essential. As with most aspects of "good laboratory practices," this is a matter of personal attitude and discipline. Planning each activity and contingency can mitigate most problems. It should also be recognized that use of isotopic markers in species or in applications that could enter human food chains is inappropriate.

Radioisotopes may not be practical for many studies or baiting situations. On the other hand, they offer advantages of multiple markers and simple assay, while minimizing much of the concern about animals changing behavior toward the bait materials as a result of the marker materials.

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