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A REVIEW OF CHEMICAL AND PARTICLE MARKING AGENTS USED FOR STUDYING VERTEBRATE PESTS

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ABSTRACT: A wide variety of chemicals including dyes, stains, inks, drugs, fluorescent and non-fluorescent particles, and radioisotopes have been used as markers to identify free-ranging mammals and birds. Markers are useful for studying: (1) home ranges, migration patterns, and population dynamics; (2) bait acceptance, palatability, and exposure of target animals via different baiting techniques for delivering toxicants, chemosterilants, or vaccines; and (3) exposure of non-target animals to control techniques. Five general classes of markers with specific marking capabilities are available for use: (1) *dyes, stains, and inks* that may be either fluorescent or non-fluorescent which stain the gastro-intestinal tract and its contents, urine, fecal droppings, or hair; (2) *inert particles*, either fluorescent or non-fluorescent, that can be detected in the gastro-intestinal tract and feces, and can be applied with an adhesive spray to birds' feathers; (3) *tetracyclines* that can be detected as a yellow fluorescence in bones and teeth; (4) *blood markers* that can be detected in the plasma or sera (e.g., iophenoxic acid or mirex); and (5) *radioisotopes* that have various patterns of tissue distribution depending upon the isotope used.

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INTRODUCTION

Several terms such as biomarker (Fletcher et al. 1990), physiological marker (Knowlton et al. 1988), seromarker (Hadidian et al. 1989), and particle marker (Fall and Johns 1988) describe marking agents that have been used to detect, trace, and identify animals. During the past 50 years hundreds of chemicals have been evaluated as markers for animals but only a small percentage have been commonly used. Some chemicals such as radioisotopes are used infrequently because of strict regulatory requirements, while other chemicals such as the tetracyclines (Linhart and Kennelly 1967, Lefebvre et al. 1987, Hanlon et al. 1989) are commonly used in a variety of animals. Chemicals for marking mammals and birds (Stonehouse 1978, Day et al. 1980), rats (Taylor and Quay 1973), and birds (Marion and Shamis 1977) have been reviewed and it is beyond the scope of this paper to present a review of all chemicals used for marking animals. Many markers were initially developed for studying free-ranging behavior of animals and only in the past 20 to 30 years have markers been incorporated into studies of vertebrate pests, usually in the form of bait markers. The objective of this review is to emphasize markers used for vertebrate pests.

Five classes of bait markers have been identified (Eason and Batcheler 1991). We have made some modifications in this classification and included uses other than bait marking for some of the materials. The five classes of markers that will be discussed are: (1) dyes, stains, and inks, (2) inert particles, (3) tetracyclines, (4) blood markers, and (5) radioisotopes.

DYES, STAINS, AND INKS

Numerous dyes and stains have been evaluated for studying the movements of small mammals (New 1959). Dyes are qualitative markers and, depending on the type, have the advantage of being detected with either the unaided eye or an ultra-violet (UV) light. A disadvantage of dyes is that most can be detected internally (gastrointestinal tract, fat) or externally (pelage, plumage, feet) for only a few days. For some applications only a short duration is required. For example, the detectability of bromocresol green, an acid-base

indicator, is less than 24 hours in the gastrointestinal tract; it was used to indicate bait acceptance by *Peromyscus maniculatus*, *Microtus ochrogaster*, and *M. pennsylvanicus* within a 24-hour interval (Nass and Hood 1969).

Rhodamine B is an intense, fluorescent dye detectable with the unaided eye at high concentrations and with a UV light at low concentrations not detectable in visible light. It is a qualitative marker of bait consumption by animals (Cowan et al. 1984). In black-tailed jackrabbits (*Lepus californicus*) a single exposure remains detectable for at least 6 weeks on the pelage, and in the gastrointestinal tract, feces, and urine for 6 to 8 days (Evans and Griffith 1973). The systemic marking capability of rhodamine B has been noted (Evans and Griffith 1973, Ellenton and Johnston 1979) and the development of fluorescent bands in claws, hair, and feathers after its ingestion has been reported in detail. The claws and hair of coyotes (*Canis latrans*) stayed marked for at least 175 days (Johns and Pan 1981), and the claws and hair of mountain beavers (*Aplodontia rufa*), pocket gophers (*Thomomys mazama*), and feathers of domestic chickens (*Gallus* sp.) remained marked for several weeks (Lindsey, 1983). The suspected carcinogenic activity of rhodamine B was discussed by Lindsey (1983). In humans there is no adequate data and only limited evidence in animals for carcinogenicity (IARC 1987).

Fluorescein is a water soluble fluorescent dye that was incorporated into an avicide formulation of 3-chloro-4-methylbenzenamine to detect birds which had been aerially sprayed in a roost (Heisterberg et al. 1990). Although aerial application has the potential to mark large numbers of birds, the usefulness of fluorescein is limited because it washes from feathers within 1 to 2 days.

Printer's ink is utilized on tracking tiles (boards) for censusing the relative population density of rodents (West et al. 1976). The efficacy of rodenticides can be assessed with this method by monitoring rodent activity before and after treatment (Poché and Mian 1986).

INERT PARTICLES

Inert particles are useful markers because they are nontoxic to animals and are chemically stable in sunlight and

under adverse weather conditions. Fluorescent and non-fluorescent particles are available and require minimal equipment for their detection. Various types of fluorescent pigments have been used to study the movements and home ranges of rodents (Frantz 1972, Lemen and Freeman 1985, Mullican 1988).

Inert particles have limited application as bait markers in pest control research because they remain in the gastrointestinal tract for only a few days after ingestion. Jones (1978) proposed the use of fluorescent pigment in a tracking dust to optimize the placement of rodenticidal dusts for control of house mice (*Mus musculus*). Johns and Thompson (1979) proposed the use of plastic particles coded with multiple colors in toxic baits to identify the toxicant in poisoned animals. Baits containing non-fluorescent, colored metallic flake particles (Fall and Johns 1987) have been evaluated in pen trials with wild Norway rats (*Rattus norvegicus*) and in field trials with roof rats (*R. rattus*). By using colored particles unique to bait sites, it was possible to determine specific feeding locations for each rat and the percent of animals feeding on the bait.

Thousands of birds can be marked from a single application using fluorescent particles formulated with an adhesive and applied by spray to bird roosts. This technique has been used to mark roosting red-winged blackbirds (*Agelaius phoeniceus*) (Otis et al. 1986, Knittle et al. 1987) and red-billed quelea (*Quelea quelea*) (Johns et al. 1989). Details of the spray technique and formulation, and a review of mass-marking in birds are provided by Johns et al. (1989). The mass-marking of birds provides greater detail of migration and flight patterns which can be used for developing control practices in agricultural and urban situations.

TETRACYCLINES

Tetracyclines are a class of antibiotics which are used as bait markers. The tetracyclines most commonly used are demethylchlortetracycline (DMCT also known as demeclocycline hydrochloride or DMCH), doxycycline monohydrate (DC), tetracycline hydrochloride (TCH), chlor-tetracycline hydrochloride (CTH), and oxytetracycline (OT). These chemicals chelate with calcium ions in bones and teeth and produce a golden-yellow fluorescent mark under UV light. Tetracyclines are effective in marking carnivores (Linhart and Kennelly 1967, Ellenton and Johnston 1979) and rodents (Crier 1970, Lefebvre et al. 1988) for several months, and marked samples can be frozen for at least 6 months without loss of fluorescence (Crier 1970). Usually the bones or teeth are removed from a carcass to observe the mark but fluorescence has been observed in the incisors of live Wistar white rats (*Rattus norvegicus*) 2 to 4 weeks after consuming DMCT (Crier 1970). DMCT has persistent and quantitative marking characteristics in the European rabbit (*Oryctolagus cuniculus*) (Cowan et al. 1984), but does not produce a detectable mark in brush-tailed possums (*Trichosurus vulpecula*) (Morgan 1981).

Some of the uses of tetracyclines in carnivores are summarized in Table 1. Linhart and Kennelly (1967) used DMCT to mark coyotes that consumed antifertility baits. All the other applications in Table 1 refer to the testing of placebo oral rabies vaccine baits for delivering rabies vaccine to target animal populations. Table 2 summarizes some of the uses of tetracyclines in rodents under laboratory or field conditions.

Table 1. Use of tetracyclines as oral bait markers in carnivores.

Species	Tetracycline used	Route ^a	Dose ^b	Reference
Coyote (<i>Canis latrans</i>)	DMCT ^c	gavage	10 mg/kg	Linhart and Kennelly 1967
	DMCT	bait	75 mg	
Red fox (<i>Vulpes vulpes</i>)	TCH ^d	diet	2-10+mg/kg	Ellenton and Johnston 1979 Bachmann et al. 1990
	TCH	diet	2-10+mg/kg	
	TCH	bait	75-475 mg	
Raccoon (<i>Procyon lotor</i>)	OT ^e	bait	100-200 mg	Hanlon et al. 1989
	TCH	bait	200 mg	Hanlon et al. (in press)
	TCH	bait	75-475 mg	
	T ^f	bait	100 mg	
	T	bait	100 mg	
Skunk (<i>Mephitis mephitis</i>)	TCH	bait	75-475 mg	Bachmann et al. 1990

^aMethod of administration to animal.

^bDose expressed as total dose (mg) or per unit of body weight (mg/kg).

^cDMCT = demethylchlortetracycline.

^dTCH = tetracycline hydrochloride.

^eOT = oxytetracycline.

^fTC = tetracycline.

Table 2. Use of tetracyclines in rodents under laboratory (L) or field (F) conditions.

Species	Tetracycline used	Route ^a	Dose ^b	Reference
Wistar rat (<i>Rattus norvegicus</i>)	DMCT ^c , DC ^d , TCH ^e	gavage	2-250 mg/kg	Crier 1970 (L)
Norway rat (<i>R. norvegicus</i>)	DMCT	bait bait	1% 0.5%	Nass et al. 1971 (F) Lindsey et al. 1971 (F)
Polynesian rat (<i>R. exulans</i>)				
Black rat (= roof rat) (<i>R. rattus</i>)	DMCH ^f	bait	1%	Lefebvre et al. 1985 (F)
Cotton rat (<i>Sigmodon hispidus</i>)	DMCH, TCH, CTH ^g	gavage	48-243 mg/kg	Lefebvre et al. 1987 (L)
Roof rat (<i>R. rattus</i>)			32-162 mg/kg	

^aMethod of administration to animal.

^bDose expressed as per unit of body weight (mg/kg) or % in bait.

^cDMCT = demethylchlortetracycline.

^dDC = doxycycline monohydrate.

^eTCH = tetracycline hydrochloride.

^fDMCH = demeclocycline hydrochloride which is another name for DMCT.

^gCTH = chlortetracycline hydrochloride.

BLOOD MARKERS

Iophenoxic acid (IA) is an organic iodine chemical that binds to proteins in the blood. It produces its "mark" by elevating the level of protein-bound iodine (PBI) which is measured by a sophisticated sensitive analytical method for iodide. This is an indirect analysis of IA. As compared to control PBI, levels of PBI after treatment with IA can be elevated several-fold in mammals for many weeks; however, IA does not produce a "mark" of any practical significance in birds (Larson et al. 1981). There is placental transfer of IA into human fetuses where it is retained in the blood of children for several years (Carakushansky 1969). This fact has not been exploited in wild mammals.

IA is used as a marker for simulated toxicant (Larson et al. 1981) and oral rabies vaccine baits (Hadidian et al. 1989, Trewhella et al. 1991). Eason and Batcheler (1991) reported that IA can be used as a quantitative marker in feral goats. Doses and additional applications of IA are summarized in Table 3.

Mirex is an organochlorine and residues are detected in the blood by gas chromatography in both mammals and birds for several weeks after ingestion (Larson et al. 1981, Knowlton et al. 1988). Although mirex can produce a "mark" for a long duration, it has the disadvantages of requiring sophisticated instruments for its analysis, being persistent in the environment, and being classified as a possible human carcinogen (IARC 1987). Buckle et al. (1987) investigated the feeding behavior of wild Norway rats (*Rattus norvegicus*) to simulated acute rodenticide bait or anticoagulant bait treatments by using four differently marked organochlorine baits. Residues of the organochlorines were detected in liver tissue by gas chromatography and could probably have been detected in blood samples also.

Sulfadimethoxine (SDM) is a broad-spectrum antimicrobial that has recently been evaluated as a blood serum marker in raccoons consuming rabies vaccine baits (Hanlon et al. in press). SDM is a short-term marker with a duration of about 7 days after a 250 mg ingested dose. Sera residues can be detected with a commercially available qualitative enzyme immunoassay card test.

RADIOISOTOPES

Radioisotopes are probably the most potent class of chemicals used to mark and identify animals (Crabtree et al. 1989) but strict licensing and use requirements by regulatory agencies effectively limits their general utilization in vertebrate pest control research. Despite the strict regulations, radioisotopes may be practical for research programs which require minute amounts of chemical. Knowlton et al. (1989) have demonstrated that penned coyotes can be marked for at least 20 days after ingesting baits containing six gamma-emitting isotopes. "Marks" from the isotopes could be detected in live animals from the abdomen and throat regions. These investigators observed a differential distribution of isotopes in tissues and provided a detailed discussion on the practical use of isotopes as marking agents.

SUMMARY

Many chemicals are available for marking vertebrate pests and each has its own unique advantages and disadvantages for a particular application. All markers must produce a detectable "mark" for the duration of a study and chemicals in baits should be palatable and well accepted. The majority of chemicals produce qualitative "marks" which are adequate for most studies. Quantitative marking characteristics have been reported for only 2 markers, demethylchlortetracycline

Table 3. Duration of mark for iophenoxic acid after oral administration in various mammals.

Species	Dose ^a	Duration of mark (weeks)	Reference
Coyote (<i>Canis latrans</i>)	5 mg 10 & 15 mg	> 8 > 16	Larson et al. 1981 Knowlton et al. 1988
Red fox (<i>Vulpes vulpes</i>)	5 mg 10 mg 20 mg	6-8 6-13 13-34	Larson et al. 1981 Baer et al. 1985
Domestic dog (<i>Canis familiaris</i>)	10 mg 20 mg	34-52 52	
Badger (<i>Taxidea taxus</i>)	5 mg	> 8	Larson et al. 1981
Raccoon (<i>Procyon lotor</i>)	5 mg	> 8	
Striped skunk (<i>Mephitis Mephitis</i>)	5 mg	> 8	
Arctic fox (<i>Alopex lagopus</i>)	20 mg	13	Foilman et al. 1987
Wild swine (<i>Sus scrofa</i>)	20 mg	> 1	Fletcher et al. 1990
Feral goat (<i>Capra sp.</i>)	1.5 mg/kg 5 & 50 mg	> 12 > 7	Eason and Batcheler 1991

^aDose expressed as total dose (mg) or per unit of body weight (mg/kg).

and iophenoxic acid. One of the greatest needs in marker research is development of inexpensive detection assays that could be used in the field. Immunological marking was proposed in 1973 by Taylor and Quy but we have not discovered references to use of this technique in wildlife baiting research. Recent advances in the bioengineering field could probably be adapted for marking animals but we are not aware of research for this purpose.

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