

Michael W. Fall¹ and Brad E. Johns¹

Metallic Flake Particle Markers for Determining the Feeding Behavior of Rats at Bait Points

REFERENCE: Fall, M. W. and Johns, B. E., "Metallic Flake Particle Markers for Determining the Feeding Behavior of Rats at Bait Points," *Vertebrate Pest Control and Management Materials: 5th Volume, ASTM STP 974*, S. A. Shumake and R. W. Bullard, Eds., American Society for Testing and Materials, Philadelphia, 1987, pp. 128-133.

ABSTRACT: Earlier work established the utility of coded particles incorporated in baits to identify animals that had recently eaten the material. Using a low-cost commercial product² (Glowble), we have extended this technique for practical field use. Laboratory, pen, and field trials were conducted to test formulations and develop methods for positive identification of marked animals under field conditions. The technique allows determination of percentages of animals feeding on bait material and of specific bait sites where individual animals have fed. Our general approach required examination of carcasses to identify marked animals. Low cost, availability of several codes, and simplicity of identifying marked animals are the principle advantages over similar marking techniques.

KEY WORDS: marker, tracer, bait, identification, rat, rodent, feeding behavior

Marking techniques provide powerful tools for field study of animal movements and feeding behavior. They also have great potential for use in simulation studies of chemical control methods and environmental exposure evaluations. A variety of materials mixed in bait have been used to mark rodents. Numerous dyes [1-3] and fluorescent pigment [4] have been used for movement studies of small mammals. Dyes, pigments, drugs, and other chemicals have been used or considered for estimating the proportions of animal populations feeding on bait material [5-9]. Problems with some of the available marking materials have included high-cost, fading, indeterminate marking periods, effects on bait acceptance, cumbersome analytical methods for field samples, and single or limited numbers of distinct identity codes. The development of Microtaggants [10] solved some of the problems associated with using coded bait. But, high cost and the need for microscopic examination limited its utility for field studies.

This paper summarizes the results of preliminary laboratory, pen, and field tests of another inert particle marker available at low cost in numerous colors that can be readily identified by examination of gastrointestinal tract contents in the field. This material, Glowble³, functions in the same manner as other particle markers, passing rapidly through the digestive tracts of animals. Our purpose in these studies was to find a material suitable to identify individual rodents among field collections that had ingested bait from specific locations. Other potential uses include studies that: (1) simulate acute toxicants, where death occurs soon after bait is ingested;

¹Supervisory wildlife biologist and research physiologist, respectively, Denver Wildlife Research Center, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, P.O. Box 25266, Denver, CO 80225-0266.

²Reference to trade names does not imply endorsement of commercial products by the federal government or its agencies.

³Registered trademark of Metallflake, Inc., Box 950, Haverhill, MA 01830.

(2) simulate chronic toxicants, where ingestion over several days may continue until just prior to death; and (3) simulate or use toxicants when examining baiting techniques for primary or secondary exposure of nontarget animals.

Description of Material

The colored particles consist of flat flakes of metalized polyester film. They were originally developed for incorporation in automotive paint finishes. Ten individual colors are available; silver appeared as a minor contaminant in other colors and should be avoided in studies requiring several codes. The colors are brilliant and highly reflective, making recognition easy in either sunlight or artificial light. The particles are rectangular in shape and somewhat variable in size. A typical particle measured 442 by 374 by 34 μm ; 1 g contained approximately 131 000 particles. The specific gravity of a particle sample was determined as 1.25. Color intensity and particle configuration appeared unaffected by digestive passage in rodents. The particles were, however, highly responsive to static electricity; considerable care was needed to avoid contamination while preparing baits. All bait formulations tested contained a particle concentration of 0.3% by weight, following Ref 10.

Bait Acceptance and Particle Identification

Formulation procedures, bait acceptance, and methods of identifying particles were developed in preliminary laboratory studies. Particles were combined with cornmeal and mixed in a metal container. Two colors, bright red and silver, were used separately, with a complete cleanup between batch preparation. Twelve white laboratory rats (two groups of six) were provided water and bait material containing one or the other of these colors *ad libitum* for three days. No alternate food was available. All animals ate normally; there was no indication that the particles affected bait acceptance. At the end of this period, fresh feces were collected and the animals were necropsied. Colored particles were readily visible through the walls of the gastrointestinal tracts. Forty-eight samples—stomach, caecum, the distal 5 cm of colon, and feces from each animal—were taken for thorough examination.

A procedure suitable for field examination of gut contents and feces was developed at this time and was used for identifying particles in subsequent trials. Contents of one sample were expressed onto the top half of a petri plate and diluted slightly with water. The bottom half of the petri plate was pressed down into this material, spreading it across the plate as a thin, translucent layer. By tipping the plate back and forth near an incandescent lamp, colored particles were easily seen. The particles were also readily visible in sunlight. All 48 samples from these rats contained particles.

Although particles could be readily identified in fecal samples, we dropped this collection from further trials because we anticipated our major field use would involve examination of recovered rat carcasses. Expression of feces from live-trapped rats before they are released offers a potential additional use of this material in behavior studies. Marking of larger mammals for which scat collection and examination are more routine than for rats offers another.

Digestive Passage

Although we anticipated from work with other particles [10] that this material would quickly pass the digestive system of a rat, a confirmation test was conducted. Four groups of six white laboratory rats each (three male, three female) were acclimated to feeding on ground laboratory rat chow for several days. Three particle colors, bright red, royal blue, and marigold, were selected and mixed with food, as previously described. Rats were placed in alternate cages on cage racks to avoid contamination from spillage. Coded bait was provided *ad libitum* for a single 24-h period. One male and one female in each group received bait containing one of the

three color codes. Following the feeding period, rats were moved to clean cage racks and maintained on laboratory rat chow. Necropsies and examinations were conducted by group at 24 h (immediately after feeding), 72 h, 168 h (one week), and 336 h (two weeks) using the procedures described.

All rats in the 24-h group were well marked throughout their tracts (Table 1). In the group examined 72 h after feeding, few particles were evident; only half these animals showed any evidence of having eaten the coded bait. Organ samples of marked animals in this group contained only one or two particles, but these were readily visible when the petri plates were tipped under light. No particles were found in samples from the groups examined one and two weeks after feeding.

Pen Trials

Two tests, about three weeks apart, were conducted in a large outdoor pen which held > 100 wild Norway rats (*Rattus norvegicus*) free feeding on laboratory rat chow. Similar procedures were used in each test, except that live traps were used to recover animals for examination after the first and snap traps (which kill animals immediately) after the second. Five particle colors were selected (bright red, royal blue, marigold, Nile green, and maroon) and formulated individually in cornmeal bait. Five bait stations, each containing one of the coded baits, were placed around the periphery of the pen and exposed for four (live trap trial) or five (snap trap trial) nights. Colors were arbitrarily placed in differing positions for the two trials. Trapping was conducted for a single night during each trial while bait material remained available.

In the first trial, eleven rats were live-trapped and examined (Table 2). All were marked by feeding at one or more bait stations. Colored particles from the different stations were easily distinguished. The greatest concentrations of particles were found in caecum samples, followed by stomach and colon samples. One animal had particles only in the colon, suggesting that it

TABLE 1—Percentages of laboratory rats marked at intervals after a single 24-h exposure to food containing metallic flake particle markers. Six rats were examined at each period.

| Time After Feeding, h | Organs Examined | | | Total (%) |
|-----------------------|-----------------|------------|-----------|-----------|
| | Stomach (%) | Caecum (%) | Colon (%) | |
| 24 | 100 | 100 | 100 | 100 |
| 72 | 33 | 50 | 17 | 50 |
| 168 (1 wk) | 0 | 0 | 0 | 0 |
| 336 (2 wk) | 0 | 0 | 0 | 0 |

TABLE 2—Percentages of eleven live-trapped wild Norway rats marked by feeding on coded bait at five stations in a pen colony.

| Bait Location Code | Stomach (%) | Caecum (%) | Colon (%) | Total (%) |
|--------------------|-------------|------------|-----------|-----------|
| Red | 27 | 82 | 18 | 91 |
| Blue | 27 | 64 | 27 | 64 |
| Gold | 9 | 64 | 64 | 64 |
| Green | 9 | 64 | 0 | 64 |
| Maroon | 0 | 27 | 9 | 27 |

had not fed at a bait station on the night it was trapped. At least ten animals (91%) had fed on bait coded with red particles. At least three had fed at the station coded maroon. Evidence from the rats recovered indicated intermediate activity at the other stations.

Because of rapid passage of particles through a rat's digestive tract, the percentages reported as feeding on coded baits represent minimums. Some rats which had fed on coded bait more than 24 h before being trapped might not be marked.

In the second trial, only five rats were recovered for examination (Table 3). Numbers of particles were again greatest in the caecum samples. All rats had taken bait from at least one of the stations; however, none had fed at the station coded gold. At least three of the animals fed on bait marked with blue particles; the other codes received intermediate activity. These trials indicated that up to five colors could be distinguished in one rat and that either snap traps or live traps were suitable for recovering animals for examination if bait was exposed concurrently with the trapping period.

Field Trial

A single field trial was conducted on Moheli Island, Federal Islamic Republic of the Comoros. This trial was a modification of procedures described for studying rat feeding patterns on bait placed on the ground and in crowns of coconut palms [11]. The availability of color coded particles allowed concurrent placement of bait in the same study area.

Packets of cornmeal bait containing either royal blue or bright red particles were prepared in small plastic bags. Bait contents averaged about 140 g per packet. A section within a large coconut plantation occupied by roof rats (*Rattus rattus*) was selected for study. The baited area measured 100 by 200 m and contained approximately 200 palms. Bait packets with red particles were placed in the crowns of every fourth palm. Similar packets containing blue particles were placed on the ground at the bases of palms at the same rate. Bait was exposed for five days before trapping commenced. Trapping was conducted over a three-day period while bait remained available. All rats examined were snap trapped in palm crowns within the baited area. Immediate field necropsies and gastrointestinal tract examinations were made as previously described, using sunlight to observe the colored particles in petri plates. The field recording procedure was shortened somewhat by scoring a rat positive for a color code at first detection; data were not recorded on the relative positions of particles.

Of 22 rats, at least 11 (50%) had fed from bait placed in palm crowns (Table 4). At least two rats (9%) had fed from bait packets on the ground. None had recently fed at both locations. These results tend to confirm concurrent radiotelemetry observations that most rat activity occurred in the palm crowns. They also suggest that longer bait exposure periods or a greater density of bait packets would be required to allow a high proportion of the rat population the opportunity to take bait. Limited rat activity at the bait packets at open sites on the ground may also reflect the type of nervous behavior observed in this species at single bait stations [12]. The

TABLE 3—Percentages of five snap-trapped wild Norway rats marked by feeding on coded bait at five stations in a pen colony.

| Bait Location Code | Stomach (%) | Caecum (%) | Colon (%) | Total (%) |
|--------------------|-------------|------------|-----------|-----------|
| Red | 0 | 40 | 20 | 40 |
| Blue | 20 | 40 | 40 | 60 |
| Gold | 0 | 0 | 0 | 0 |
| Green | 40 | 40 | 40 | 40 |
| Maroon | 20 | 20 | 20 | 20 |

TABLE 4—Consumption of marked bait by 22 roof rats snap trapped in coconut palm crowns on Moheli Island. Cornmeal baits containing red particles were placed in palm crowns; bait packets containing blue particles were placed on the ground. Trapping commenced five days after bait placement.

| Marked Rats | Number | Percent |
|----------------|--------|---------|
| Red particles | 11 | 50 |
| Blue particles | 2 | 9 |
| Both colors | 0 | 0 |

trial confirmed the practicality of the Glowble particle markers and the examination procedures for field use.

Discussion

We have demonstrated the practical use of a low-cost, commercially available marker with several color codes for laboratory, pen, and field studies where information on feeding locations or feeding upon particular bait materials is required. A number of other somewhat similar particulate materials, known generically as "glitter," may have the same utility. Some products are available with greater numbers of colors for possible use as codes. Without data, we speculate that the larger, flat, film-based particles, such as Glowble, are more easily identifiable and less subject to digestive tract disturbance than the smaller foil-based particles.

The 0.3% particle concentration was selected solely on the basis of prior work with similar size particles [10]. Further work is undoubtedly needed to determine concentrations appropriate for different types of studies and with different species.

A serious limitation of this material, and probably of most other particle markers, is the need to design studies so that animals are collected within a short time after feeding. Longer lasting, multiple code markers that overcome the problems with existing materials are still very much needed.

Acknowledgments

We thank G. K. LaVoie, L. A. Fiedler, and K. A. Crane for assistance with laboratory work and D. C. Evans and Charrafane Abdou, who helped conduct the field trial on Moheli. D. J. Elias and anonymous ASTM reviewers provided helpful comments on the manuscript. This study was conducted under the guidance and support of the U.S. Fish and Wildlife Service, with partial funding provided by the Agency for International Development under PASA ID/TAB-473-1-67.

References

- [1] Davis, D. E., Emlen, J. T., and Stokes, A. W., *Journal of Mammalogy*. Vol. 29, No. 3, 1948, pp. 207-225.
- [2] New, J. G., *Journal of Mammalogy*. Vol. 39, No. 3, 1958, pp. 416-429.
- [3] Gast, J. A., *Ecology*. Vol. 44, No. 3, 1963, pp. 611-612.
- [4] Frantz, S. C., *Journal of Mammalogy*. Vol. 53, No. 1, 1972, pp. 218-223.
- [5] Nass, R. D. and Hood, G. A., *Journal of Wildlife Management*. Vol. 33, No. 3, 1969, pp. 584-588.
- [6] Crier, J. K., *Journal of Wildlife Management*. Vol. 34, No. 4, 1970, pp. 829-834.
- [7] Evans, J. and Griffith, R. E., Jr., *Journal of Wildlife Management*. Vol. 37, No. 1, 1973, pp. 73-81.
- [8] Larson, G. E., Savarie, P. J., and Okuno, I., *Journal of Wildlife Management*. Vol. 45, No. 4, 1981, pp. 1073-1077.

- [9] Joins, B. E. and Pan, H. P., *Vertebrate Pest Control and Management Materials: Third Conference, ASTM STP 752*, E. W. Schafer, Jr. and C. R. Walker, Eds., American Society for Testing and Materials, Philadelphia, 1981, pp. 86-93.
- [10] Johns, B. E. and Thompson, R. D., *Avian and Mammalian Wildlife Toxicology. ASTM STP 693*, E. E. Kenaga, Ed., American Society for Testing and Materials, Philadelphia, 1979, pp. 80-88.
- [11] Fiedler, L. A., Fall, M. W., and Reidinger, R. F., Jr., *Proceedings Tenth Vertebrate Pest Conference*, R. E. Marsh, Ed., University of California, Davis, 1982, pp. 73-79.
- [12] West, R. R., Fall, M. W., and Libay, J. L., *The Philippine Agriculturist*, Vol. 59, No. 1 and 2, 1975, pp. 31-36.

| | | |
|----|----|----------------|
| 50 | 11 | Red particles |
| 0 | 3 | Blue particles |
| 0 | 0 | Both colors |

trial confirmed the practicality of the Gluebic particle markers and the examination procedures for field use.

Discussion

We have demonstrated the practical use of a low-cost, commercially available marker with several color codes for laboratory, pen, and field studies where information on feeding locations or feeding upon particular bait materials is required. A number of other somewhat similar polyethylene materials, known generically as "glitters", may have the same utility. Some products are available with greater numbers of colors for possible use as codes. Without data, we speculate that the larger, flat, film-based particles, such as Gluebic, are more easily identifiable and less subject to disruptive tract disturbance than the smaller foil-based particles.

The 0.3% particle concentration was selected solely on the basis of prior work with similar size particles [10]. Further work is undoubtedly needed to determine concentrations appropriate for different types of studies and with different species.

A serious limitation of this material, and probably of most other particle markers, is the need to design studies so that animals are collected within a short time after feeding. Longer feeding periods code markers that overcome the problems with existing materials are still very much needed.

Acknowledgments

We thank D. E. Layton, L. A. Fiedler, and K. A. Cruise for assistance with laboratory work and D. C. Evans and Charles Arden, who helped conduct the field trial on Malsb. D. J. Bliss and anonymous ASIS reviewers provided helpful comments on the manuscript. This study was conducted under the guidance and support of the U.S. Fish and Wildlife Service with partial funding provided by the Agency for International Development under FASA IDA TAB 473-1-01.

References

- [1] Davis, D. E., Emlen, J. T., and Zisler, A. W., *Journal of Mammalogy*, Vol. 57, No. 3, 1976, pp. 507-512.
- [2] New, L. O., *Journal of Mammalogy*, Vol. 59, No. 3, 1978, pp. 416-427.
- [3] Galt, J. A., *Ecology*, Vol. 44, No. 3, 1963, pp. 611-612.
- [4] Evans, D. C., *Journal of Mammalogy*, Vol. 57, No. 1, 1976, pp. 218-222.
- [5] Marx, R. D. and Hunt, G. A., *Journal of Wildlife Management*, Vol. 37, No. 3, 1966, pp. 584-588.
- [6] Frost, K., *Journal of Wildlife Management*, Vol. 34, No. 4, 1970, pp. 529-534.
- [7] Evans, J. and Gilbert, R. E., *Journal of Wildlife Management*, Vol. 37, No. 1, 1973, pp. 33-61.
- [8] Larson, G. E., Cavart, F. J., and Orsino, J., *Journal of Wildlife Management*, Vol. 42, No. 4, 1978, pp. 1047-1051.