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Tetracyclines as Fluorescent Bone Markers in Cotton and Roof Rats

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ABSTRACT: Three tetracycline compounds, demeclocycline hydrochloride (DMCH), tetracycline hydrochloride (TCH), and chlortetracycline hydrochloride (CTH), were administered by gavage and tested as fluorescent bone markers for adult cotton *(Sigmodon hispidus)* and roof *(Rattus rattus)* rats. Probit and logistic regression models did not fit the data well; a more extensive test, with more widely spaced doses and additional low doses, may improve fit. TCH and CTH, which are considerably less expensive than DMCH, appeared to be similar to DMCH in marking roof rats at higher doses (72, 108, and 162 mg/kg) but not at low doses (32 and 48 mg/kg). Cotton rat mandibles were not as distinctly marked as those of roof rats by any of the compounds. Only at high doses (162 and 243 mg/kg) of DMCH or TCH were more than 50% of cotton rats scored as marked by both evaluators. The dose-response test results support field evidence that DMCH is not an effective marker for cotton rats. Neither TCH or CTH appear to be better candidates than DMCH as markers for cotton rats.

KEY WORDS: cotton rat *(Sigmodon hispidus),* roof rat *(Rattus rattus),* fluorescent markers, demeclocycline, tetracycline, chlortetracycline, dose-response

Tetracyclines have been used successfully in several wildlife field studies to detect the percentages of animals that consume baits and to trace movements of individuals into or out of baited areas [*1–4*]. Tetracycline compounds bind with calcium in bone to induce a yellow-gold fluorescence visible under ultraviolet (UV) light [*5*]. Crier [*2*] tested the efficacy of three tetracyclines in marking adult Wistar albino rats and concluded that demethylchlortetracycline (DMCT) was superior to the other two compounds tested [doxycycline monohydrate and tetracycline hydrochloride (TCH)]. Demethylchlortetracycline has been renamed demeclocycline hydrochloride (DMCH) [*6*].

DMCH was four times more expensive than TCH at the time of Crier's [*2*] test. Even in bulk, DMCH is three to four times more expensive than CTH (chlortetracycline hydrochloride) or TCH. The cost of DMCH capsules is greater than 20 times the cost of TCH capsules, and TCH is more readily available than DMCH. After finding that DMCH (1% on oat groats) was an effective marker in a roof rat field study [*7*], we wanted to determine if a less expensive related chemical would provide comparable marking efficacy. We noted in the same study that DMCH did not appear to be an effective marker for cotton rats; only 27% (11/41) of cotton rats captured in baited areas showed fluorescence. Other field (N. R. Holler, U.S. Fish and Wildlife

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Service, unpublished data) and laboratory data [8] indicate that cotton rats readily accept oat groat baits.

We are reporting results of comparative efficacy testing of DMCH, TCH, and CTH to determine: (1) if either is an acceptable, less expensive substitute for DMCH for marking roof rats; and (2) if laboratory testing supports field results that indicate DMCH is not an effective marker for cotton rats. We also suggest several ways to improve our dose-response test for evaluating fluorescent rodent markers.

Methods

Adult cotton and roof rats, trapped in South Florida sugarcane fields, were maintained in individual cages for at least three months prior to testing in August 1983. Rats were fasted approximately 18 h prior to administration of the chemicals by gavage. Five doses were tested for each of the three tetracycline compounds. Cotton rats received doses of 48, 72, 108, 162, and 243 mg chemical/kg body weight; roof rats received doses of 32, 48, 72, 108, and 162 mg/kg. (Cotton rats received higher doses because field results with DMCH indicated poorer marking in this species.) Four or five cotton rats (total = 67) and five roof rats (total = 75) were randomly assigned to each dose level, with two to three of each sex/dose. Tetracycline compounds were dissolved or suspended in 2% Methocel solution, and doses were administered at a constant volume of 1 mL by gavage with a syringe and ball-tipped needle.

Six days after dosing, rats were sacrificed and mandibles were extracted and scraped clean. Two experienced evaluators (A and B), blind to compound and dosage, independently examined the mandibles under long-wave UV light (3600 to 3700 Å) and scored each as positive or negative for fluorescence. Mandibles of rats that had not received a tetracycline compound were available for reference. The proportions of fluorescent/nonfluorescent mandibles were determined for each treatment by species by evaluator combination.

Two types of dose-response analyses were used on the data: probit analysis and logistic regression. Both statistical techniques model a function of the probability of seeing a treatment effect (in this case, fluorescence) in terms of the dose given. The data were analyzed using PROC PROBIT and PROC FUNCAT (for the logistic regression) in the Statistical Analysis System (SAS) software package [9]. For the probit analysis the proportions of mandibles recorded as fluorescent by each evaluator were averaged at each dose level for the different treatments and species. In the FUNCAT analysis a logistic regression model was fit for each treatment by species by evaluator combination.

Results and Discussion

The probit model approach did not work well for any of the three treatment markers in either species. In five of the six data sets, upper or lower fiducial limits could not be estimated for all doses; when they could be estimated, the ranges were generally too large to be useful. The only data set for which these limits could be estimated for all doses was that for the CTH marker on roof rats. A CTH dose of 128 [95% fiducial limits (FL) = 87 to 571] mg/kg was estimated to produce detectable fluorescence in 90% of an exposed population. Doses of 91 (95% FL = 65 to 220) mg/kg would produce a mark in 75% of exposed animals; doses of 63 (95% FL = 39 to 65) mg/kg would mark approximately 50% of exposed animals.

The logistic regression models also tended to fit the data poorly. Dosage was found to have a significant effect ($P < 0.05$) on the probability of detecting fluorescence in 2 of the 12 fitted models. These two were from each evaluator's data on CTH used on roof rats [$\chi^2 = 6.44$ and 4.02, 1 degree of freedom (df) each]. Although the possibility of obtaining 2 random false significance values out of 12 tests exists (about a 10% chance at the 0.05 level), the fact that they were both found in CTH roof rat data (by the two observers) tends to support acceptance of a significant dose-response effect of CTH on roof rats. It also is interesting to note that the dose effect of

TCH on roof rats approached significance in the logistic regression models of both evaluators' data ($\chi^2 = 3.09$ and 2.73, 1 df each, $0.05 < P < 0.10$).

Several factors, singly or in combination, may explain the poor fit and lack of significant dose-response in the two model types. First, the dose-response was similar at many dose levels, as with DMCH-treated roof rats (Fig. 1). Second, results were inconsistent, such as CTH-treated cotton rats showing low responses at some high doses and high responses at some low doses (Fig. 1). Third, the sample size was small. The study design could be improved by increasing the number of rats/dose and perhaps by increasing the difference between dose levels. A lower dose was probably needed for most of the treatment by species trials. In the case of DMCH on roof rats, at least two lower dose levels were needed, as marking was better than anticipated even at 32 mg/kg. The subjectivity in scoring of mandibles for fluorescence undoubtedly contributed to the apparent inconsistencies in dose response. However, the fluorescent response varied among rats that received the same dose. The use of more than one evaluator is important in assigning a subjective score to give some idea of error and bias inherent in the technique.

Although rigorous evaluation of differences among treatments is not possible, several tentative conclusions can be offered. DMCH appeared to be more effective in marking roof rats at low doses (≤ 48 mg/kg) than TCH or CTH, by both evaluators' scores (Fig. 1). DMCH marked roof rats well (min = 80%) at all doses tested, but DMCH did not appear to be as effective in marking cotton rats, particularly at low doses. TCH and CTH did not appear to differ greatly

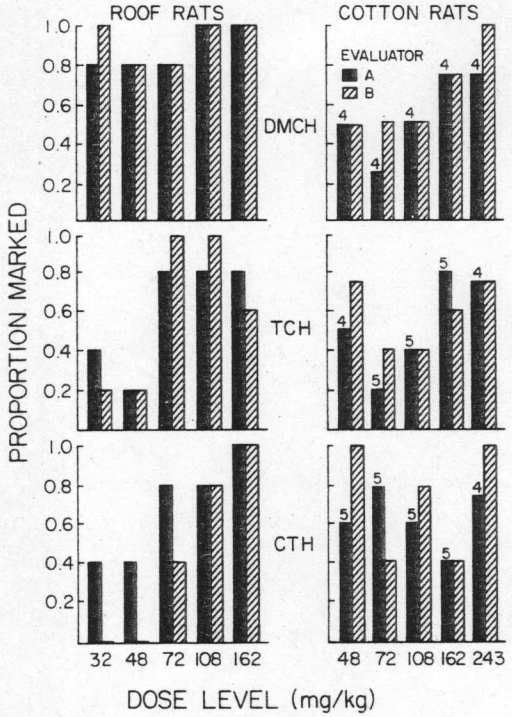

FIG. 1—*Proportions of roof and cotton rat mandibles determined to show fluorescence by Evaluators A and B after oral dosing of three tetracycline compounds, by dose level. DMCH = demeclocycline hydrochloride, TCH = tetracycline hydrochloride, CTH = chlortetracycline hydrochloride. Doses were administered to five roof rats and four or five cotton rats per tetracycline/level (the number of cotton rats dosed appears at the top of Evaluator A's bars on the cotton rat graphs).*

from DMCH in marking roof rats at higher doses ($\geq$ 72 mg/kg), although Evaluator B detected fluorescence in only 60% of the roof rats that received the highest dose of TCH (Fig. 1).

There were fewer disagreements between the evaluators in scoring mandibles marked by DMCH (5 of 45 were scored differently by the evaluators) than TCH (9 of 48) or CTH (13 of 49). Disagreements in scoring of cotton rat mandibles totaled 14 of 67 (21%), and 13 of 75 (17%) roof rat mandibles were scored differently. Evaluator A tended to have a negative bias in scoring cotton rat mandibles compared to Evaluator B; of 14 disagreements, A scored 10 negative and 4 positive. In scoring roof rat mandibles, Evaluator A had a positive bias; of 13 disagreements, A scored 9 positive and 4 negative. This reversal in scoring tendency is probably related to a difference in the pattern of marking between cotton and roof rat mandibles. Roof rats' mandibles tended to have bright spots of fluorescence, even if only in one or two places. The mandibular condyle, coronoid process, and base of the incisors were the most frequently marked areas on the mandible. Similar marking patterns were noted in Wistar rats [*2*]. Cotton rat mandibles were less distinctly marked, showing much less intense fluorescence, often only a pale yellow cast over a large area of the mandible. Intense spots of fluorescence were rarely noted; in two animals, one or more molars were well marked. The results of the laboratory testing appear to support the difference in DMCH marking of cotton and roof rats noted in the field. Only at high doses (162 and 243 mg/kg) were more than 50% of the cotton rats scored as marked by both evaluators.

Crier [*2*] concluded that TCH adequately marked Wistar rats at 250 mg/kg but not at 50 mg/kg and that DMCH was superior in marking effect to TCH. He did not, however, test doses between 50 and 250 mg/kg, or between tetracyclines at each dose. The differences he reported between DMCH and TCH at 250 mg/kg were very slight. Also, the rating scale used by Crier combined quantitative (% marked) and qualitative (amount and intensity of fluorescence) scores, making the interpretation of different ratings difficult. The methods used in this study are an attempt to minimize possible bias in an essentially subjective evaluation. The results, while tentative, indicate that intermediate doses of DMCH and TCH (72, 108, 162 mg/kg) did not appear to differ in their marking of roof rats. Low doses of DMCH, however, appear to be more effective than TCH or CTH in marking roof rats, and DMCH-induced fluorescence was more consistently detected by both evaluators.

Both TCH and CTH may be acceptable substitutes for DMCH to mark roof rats in some research situations, although bait acceptance studies should be conducted prior to field use of these alternatives. Bait acceptance by European rabbits *(Oryctolagus cuniculus)* was markedly reduced by DMCH [*10*], and CTH is described as "bitter" [*6*]. If bait acceptance is not a problem, much more TCH and CTH than DMCH could be economically used in a field application by increasing the amount/bait particle, the amount of bait used, or both. When it is important to optimize detection of low levels of bait ingestion, DMCH may be the better choice, even if more expensive. Neither TCH nor CTH appears to be a better marker candidate than DMCH for cotton rats.

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