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Source: Journal of Wildlife Diseases, 41(3) : 549-558

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-41.3.549

Journal of Wildlife Diseases, 41(3), 2005, pp. 549–558

EVALUATION AND SIGNIFICANCE OF TETRACYCLINE STABILITY IN RABIES VACCINE BAITS

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ABSTRACT: Tetracycline is widely used as a biomarker for bait consumption by wildlife; tetracycline is incorporated into bones and teeth and can be detected by fluorescence microscopy several weeks postconsumption. During 2003, the United States Department of Agriculture distributed more than 10 million tetracycline-containing rabies-vaccine baits to control the spread of wildlife vectored rabies to humans, pets, and livestock. To estimate the percentage of target species consuming the baits, raccoons and skunks were collected in baited areas and teeth were analyzed for the presence of the biomarker. Several incidents of low biomarker detection rates prompted an investigation of the stability of the biomarker in the baits. Baits were collected at several points along the manufacturing and distribution chain. Baits were analyzed for free and polymer-bound tetracycline and the less active isomer epitetracycline. Results indicated that a portion of the tetracycline was converted to epitetracycline. Additionally, significant quantities of both compounds were trapped in the polymer, which is homogeneously distributed throughout the bait. The results of this study suggest that approximately 40% of the target quantity of tetracycline was unavailable for absorption. This situation could contribute to low biomarker detection rates and suggests that formulation modification should be considered.
Key words: Baits, biomarker, rabies, raccoons, tetracycline, vaccine

INTRODUCTION

Tetracyclines are widely used as biomarkers for monitoring vaccine and contraceptive bait consumption in a variety of carnivores and rodents (Linhart and Kennelly, 1967; Crier, 1970; Cowan et al., 1984; Lefebvre et al., 1988; Hanlon et al., 1989; Perry et al., 1989; Bachman et al., 1990; Savarie et al., 1992; Olson et al., 2000; Rosatte and Lawson, 2001). Target species include coyotes (*Canis latrans*), feral pigs (*Sus scrofa*), mongoose (*Herpestes javanicus*), red fox (*Vulpes vulpes*), gray fox (*Urocyon cinereoargenteus*), raccoons (*Procyon lotor*), skunks (*Mephitis mephitis, Spilogale putorius*), and opossum (*Didelphis virginianus*). Following absorption, tetracycline is incorporated in calcific tissues of mammals. The tetracycline biomarker deposits can be observed following tissue sectioning using fluorescence and ultraviolet microscopy. When administered via intraperitoneal injection, tetracycline can be detected as soon as 12 hr postexposure (Milch et al., 1957). When administered orally, tetracycline can be detected as early as 2 days postconsumption (Hanlon, et al., 1989). Previously published tetracycline biomarker studies have assumed that tetracycline baits were 100% effective in biomarking animals that ingested the baits; the uncorrected frequency of biomarker detection in sampled animals was accepted as the proportion of the exposed populations that consumed the baits. Additionally, no published tetracycline biomarker studies evaluated tetracycline content or stability of the marker compounds in baits; all studies assumed the composition of the baits were directly proportional to the ratio of raw ingredients and that the instability of the biomarker compound during manufacturing and storage was insignificant. However, degradation and nonuniform distribution of tetracycline in baits could negatively impact marking efficiency. If marking efficiency of tetracycline baits is less than 100%, then the observed portion of marked animals observed in field studies should be divided by the marking efficiency to yield an accurate estimate of the portion of animals that ingested baits.

Since the 1940s, rabies vaccination programs in the USA have resulted in a decrease in rabies prevalence in dogs (Tierkel, 1975). During this time, there has been a significant increase in the prevalence of rabies detected in wild animals (Winkler, 1986; Krebs et al., 2002; Jones et al., 2003). Although the majority of human rabies exposures are due to domestic animals, wild animals constitute a significant reservoir (Hemlick, 1983; Gordon et al., 2004). In the USA, the most common wildlife species reported with rabies are skunks, raccoons, bats (many species), and red and gray fox. In the mid-1950s, an epizootic of raccoon rabies was first detected in Florida (McLean, 1975). Unlike most wildlife rabies populations, raccoon rabies occurs in areas with dense human populations as well as rural areas. This outbreak has spread north, and rabies-infected raccoons have been detected in all states of the eastern USA (Center for Disease Control, 1986).

In an effort to mitigate the western migration of terrestrial wildlife-vectored rabies in the USA, the US Department of Agriculture/Wildlife Services Program (USDA/WS) has distributed more than 40 million vaccine baits, including 10.5 million rabies vaccine baits in 2003 (Kuehn, 2002; Anonymous, 2003). These baits consist of a sachet containing oral rabies vaccine surrounded by a fishmeal polymer. To permit a cost-effective means of monitoring bait consumption by target and nontarget species, the polymer was formulated to contain 1% tetracycline (Fig. 1) as a biomarker. As an indicator of bait consumption by raccoons, the first premolar is removed for analysis of tetracycline deposits. In some instances, animals also were tested for rabies virus neutralizing antibodies.

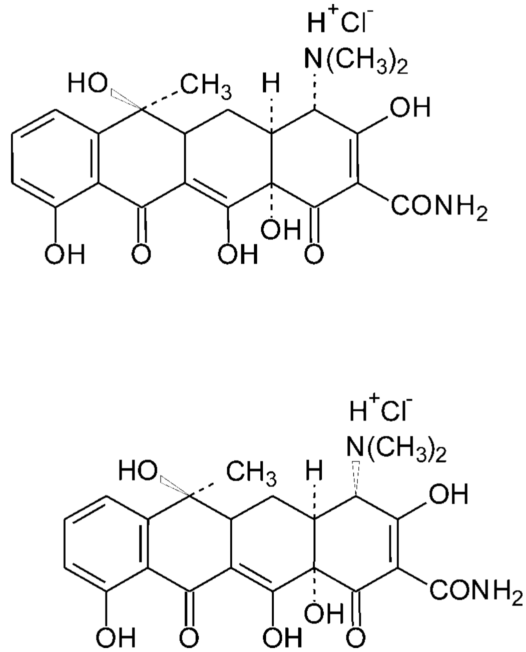

FIGURE 1. Tetracycline, epitetracycline.

Following a recent rabies vaccine bait application in Ohio, antibody analyses indicated that 40% of raccoons in the bait zone were antibody positive, yet only 10% of the population was positive for the tetracycline biomarker (Slate, 2003). Concern over this discrepancy led to the initiation of this study in which the tetracycline content of baits was quantified at various points along the manufacturing and distribution supply chain. The marking efficiency of epitetracycline, a tetracycline degradation product formed during bait manufacturing, was determined by dosing wild caught raccoons with tetracycline or epitetracycline and subsequently monitoring the first premolar for tetracycline deposits.

MATERIALS AND METHODS

Materials

Analytical-grade tetracycline hydrochloride and epitetracycline hydrochloride were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Product-grade tetracycline was obtained from Bait Tek (Beaumont, Texas, USA). Dichloromethane, acetonitrile, and phosphoric acid (H_3PO_4) were obtained from

TABLE 1. High-performance liquid chromatography (HPCL) conditions.

Mobile phase	0.05 N H_3PO_4:ACN (80:20)
Flow rate	0.5 ml/min
Oven temperature	Ambient
HPLC column	Polymer Laboratories (Amherst, Massachusetts, USA), PLRP-S 150×4.6 mm i.d., 5-μm particle size
Injection volume	10 μl
Absorbance	365 nm
HPLC run time	10 min

[a] ACN = acetonitrile; PLRP-S = Polymer Labs Reversed Phase Small; i.d. = interior diameter.

Fisher Scientific (Fair Lawn, New Jersey, USA). Corn syrup (Karo®, ACH Food Co, Memphis, Tennessee, USA) was obtained locally.

Technical analysis

The technical tetracyclines (analytical standards and raw material used for manufacturing) were dissolved in 0.05N H_3PO_4:acetonitrile (8:2) water at 50 μg/ml. Aliquots (10 μl) were analyzed by high-performance liquid chromatography (HPLC) using the conditions summarized in Table 1.

Bait analysis

Oral rabies vaccine baits were obtained from Merial, Inc. (Athens, Georgia, USA). The baits consisted of a 15-g bait block (76% fishmeal, 8%fish oil, ≈15% polymer, 1% tetracycline, <1% mold inhibitor) into which a 2-ml vaccine sachet was inserted. The rabies vaccine sachet was removed, and the bait block was homogenized by grinding in a Spex CertiPrep Freezer Mill (Metuchen, New Jersey, USA). To solubilize the plastic polymer in the baits, 4 ml dichloromethane was added to a 50-ml glass tube containing a 0.5-g aliquot of the homogenized bait matrix. While protecting the solution from light (tetracycline is photolabile), the tubes were mixed for several seconds using a vortex mixer and then placed in a sonicator for 15 min. Tetracyclines were recovered by adding 20-ml extraction solution (0.05 N H_3PO_4:methanol [8:2]) and subsequent high-speed mechanical mixing for 10 min. The tubes were again placed in a sonicator for 10 min, followed by centrifugation at 6,000 × G for 5 min. A 1.0-ml aliquot of this solution was transferred to a 10-ml volumetric flask, which was brought to volume with HPLC mobile phase. Before HPLC analysis, the diluted extraction solution was eluted through a 0.45-μm filter. The tetracycline and epitetracycline in the diluted extract were separated by HPLC and quantified versus chromatographic response (UV absorbance at 365 nm) of external tetracycline and epitetracycline standards analyzed under identical conditions (Table 1) using a Hewlett Packard (Palo Alto, California, USA) 1050 high-performance liquid chromatograph equipped with a diode-array detector (Fig. 2). The retention times of tetracycline and epitetracycline were approximately 6.25 and 7.25 min, respectively (Fig. 3).

Baits were also analyzed without the addition of the dichloromethane step. This yielded the quantity of "free" tetracyclines (i.e., tetracyclines that were not contained in the polymer matrix). Polymer-bound tetracyclines were calculated as the difference between the total and the free tetracycline content of the baits.

Before the analysis of any bait samples, the analytical methodology was validated by evaluating the recoveries of tetracycline and epite-

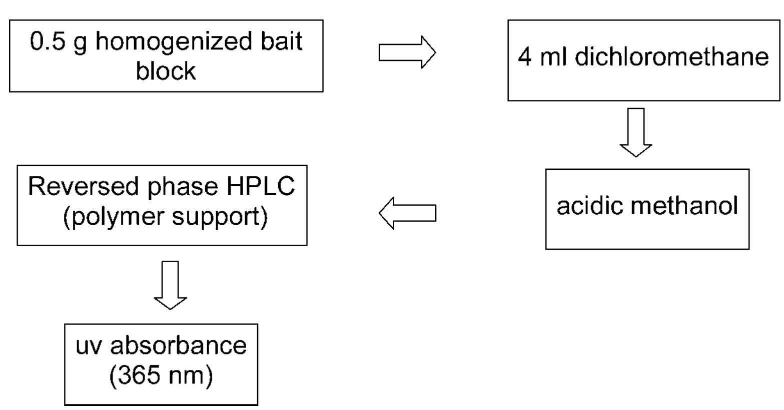

FIGURE 2. Analytical scheme for the analysis of tetracycline and epitetracycline in rabies vaccine baits.

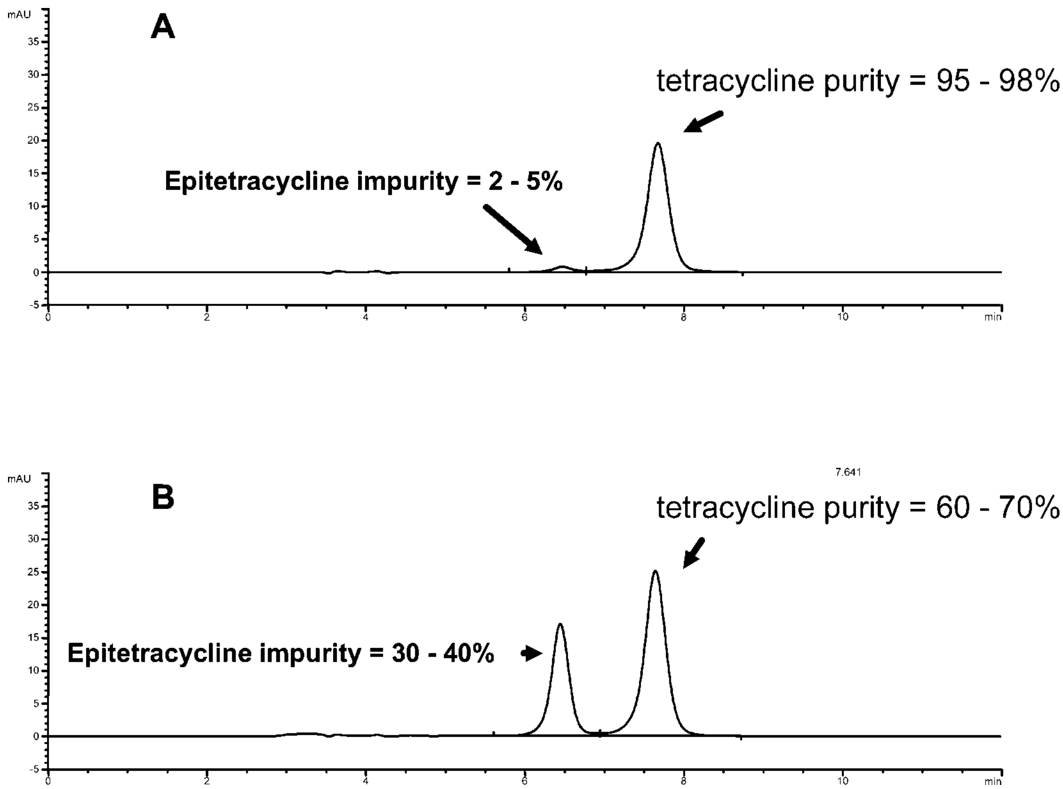

FIGURE 3. HPLC chromatograms for the analysis of tetracyclines. A: Product-grade tetracycline used for bait manufacturing. B: Manufactured rabies vaccine bait.

tracycline from laboratory-fortified control baits (no tetracycline) obtained from Bait Tek. Control baits were fortified with tetracycline at 0.5% or 1.5% and epitetracycline at 0.1% or 0.5%. Seven replicates were analyzed at each fortification level. Additionally, seven nonfortified control baits were analyzed to evaluate for the presence of coeluting compounds, which could potentially interfere with the quantification of tetracycline or epitetracycline. The method limit of detection (MLOD) was estimated as the quantity of analyte required to generate a chromatographic signal equivalent to three times the baseline noise at the retention time of tetracycline or epitetracycline in the nonfortified control sample chromatograms; this calculation was based on the mean chromatographic response factors observed for the control samples fortified with 0.5% tetracycline or 0.1% epitetracycline. The linearity of the chromatographic system over the analyte ranges of interest were determined by the duplicate analyses of five concentrations of analytical standards ranging from 10 μg to 111 μg tetracycline/ml and from 1 μg to 22 μg epitetracycline/ml.

Environmental degradation

The potential for environmental degradation of tetracyclines in the baits was estimated by placing baits in an environmental chamber (Conviron, Winnipeg, Canada). Baits were exposed to a 12:12 light:dark cycle at 30 C and 80% relative humidity. Baits were removed after 1, 3, and 7 days and were stored at −20 C for <2 wk before analysis. To estimate the potential for water leaching of tetracycline in baits exposed to rain, baits were soaked for 1 hr. The baits and water were immediately analyzed to quantify the tetracycline content.

Marking evaluation

Thirty raccoons were live-trapped in Larimer County, Colorado (USA), quarantined, and acclimated to the outdoor pen facility at the USDA/WS/National Wildlife Research Center, Fort Collins, Colorado (USA). Raccoons were maintained on daily rations of approximately 150 g of Purina Omnivore Chow (St. Louis, Missouri, USA) and ad libitum access to water. Raccoons were randomly divided into three treatment groups of 10 animals each. Each treatment consisted of a mix of male and female raccoons ranging in age from 1-yr to 5-yr old. Raccoons were fasted overnight and conditioned to consume 20 ml of 50:50 corn syrup: water between 9 am and 11 am. Food was then provided for the remainder of the day. On the day of treatment, raccoons consumed one of three treatments: 20 ml of diluted corn syrup (control); 20 ml of diluted corn syrup containing 150 mg of tetracycline (>98% pure); or 20 ml of diluted corn syrup containing 150 mg of epitetracycline (>98% pure).

Seven weeks posttreatment, raccoons were sedated with 0.1 mg/kg of ketamine:xylazine (1: 5) and a first premolar was removed from each animal. A sectioning saw was used to prepare 100-μm sections from whole teeth, and sections were permanently mounted on glass microscope slides. Tooth sections were analyzed via fluorescence microscopy for the presence of the tetracycline biomarker. Cementum aging analysis was also conducted on the tooth sections to determine the age of the donor animals (Johnston and Watt, 1981; Matson, 2003).

Statistical analyses

For validation of analytical methodology, mean recoveries were calculated for tetracycline and epitetracycline at each fortification level. Linearity of chromatographic response for each analyte was evaluated by linear regression analysis of chromatographic peak area versus analyte concentration. The mean fraction of animals marked (marking efficiency) for tetracycline- and epitetracycline-dosed raccoons were compared using Student's t-test. The Chi-square test was used to test for a significant relationship between age class and marking efficiency. Raccoon-marking data (yes/no) were analyzed by age class (1-, 2-, and 3- to 5-yr old) (Anderson, 1987).

RESULTS

Analytical method validation

Linear regression analysis of chromatographic peak response versus analyte concentration yielded r^2 values greater than 0.999 for tetracycline and epitetracycline Analysis of the control baits indicated that there were no detectable matrix-derived interferences at the retention times of tetracycline or epitetracycline. The method limits of detection were equivalent to 0.014% tetracycline and 0.011% epitetracycline. Mean analyte recoveries were 87.4% (SD = 0.88) and 90.0% (SD = 1.12) for tetracycline bait concentrations of 0.5% and 1.5% respectively. Mean recoveries were 83.1% (SD = 1.94) and 80.6% (SD = 2.51) for epitetracycline bait concentrations of 0.1% and 0.5%, respectively (Table 2).

TABLE 2. Analytical method validation recoveries.

	Tetracycline		Epitetracycline	
Sample	0.50%	1.50%	0.10%	0.50%
1	87.0	90.0	85.8	78.9
2	88.4	91.1	81.7	78.4
3	88.7	91.9	81.0	78.2
4	86.4	89.2	84.0	82.3
5	87.0	89.0	81.0	80.1
6	86.6	89.0	83.2	80.8
7	87.4	89.7	85.0	85.2
Mean	87.4	90.0	83.1	80.6
SD	0.88	1.12	1.94	2.51
CV	1.0%	1.2%	2.3%	3.1%

Sample analyses

The tetracycline and epitetracycline analytical standards were >98% pure. The purity of the tetracycline material used in the preparation of the rabies baits ranged from 95% to 98%. Epitetracycline was the only other compound detected in this material. This product material consisted of 2% to 5% epitetracycline. The mean concentration of total tetracyclines in the baits was 1.07% (SD = 0.25). However the tetracyclines in these baits consisted of 60% to 70% tetracycline and 30% to 40% epitetracycline (Fig. 3). When baits were analyzed without the addition of dichloromethane, tetracycline and epitetracycline recoveries were reduced by an average of 20.5% and 49.8%, respectively. During exposure to simulated environmental conditions, the mean epitetracycline fraction of tetracyclines in the baits increased from 39.5% (SD = 0.41) to 44.0% (SD = 0.45) over the first 3 days and to 45.7% (SD = 0.48) over the next 4 days. After soaking the baits in water for hours, an average of 2.1% of the tetracyclines were detected in the water. Epitetracycline comprised an average of 27.5% (SD = 0.32) of the total

TABLE 3. Tetracycline and age-analysis results.

Tetracycline		Epitetracycline	
Age (years)	Tetracycline analysis[a]	Age (yr)	Tetracycline analysis
2	+	5	+
2	0	1	+
3	+	2	+
3	+	2	0
1	+	4	0
1	+	2	0
1	+	2	0
1	+	2	+
		1	0

[a] + = marker positive; 0 = marker negative.

tetracycline content that leached from the baits into the water.

Marking evaluation

During the tooth analysis procedure, it was revealed that two teeth from the tetracycline-exposed group and one tooth from the epitetracycline-exposed group were damaged so as to prohibit analysis. The remaining teeth were successfully analyzed for the presence of tetracycline deposits and age determination. Ages of raccoons used in this study ranged from 1-yr to 3-yr old for the tetracycline-exposed group and from 1-yr to 5-yr old for the epitetracycline-exposed group. Tetracycline deposits were detected in seven of the eight animals successfully analyzed in the tetracycline-exposed group and in four out of nine animals in the epitetracycline-exposed group (Table 3). All tetracycline deposits appeared to be formed during the past year. The Student's t-test indicated that the fraction of animals marked in the tetracycline group was significantly greater than the fraction marked in the epitetracycline group ($P = 0.03$). The Chi-square analysis indicated that age class did not have a significant effect on the probability of detecting tetracycline deposits in the teeth of tetracycline- or epitetracycline-exposed raccoons groups ($P = 0.998$).

DISCUSSION

Vaccine baits were prepared and distributed by several entities (Fig. 4). Fish meal polymer baits were prepared by Bait Tek via heat extrusion of the bait matrix containing fish meal, plastic polymer, tetracycline, and a mold inhibitor. A sachet containing 2 ml of oral rabies vaccine was inserted into each bait block and sealed with paraffin by Merial (Harlow, Essex, UK). The vaccine baits were then shipped to various locations in the eastern USA for aerial, truck, and hand distribution by USDA/WS.

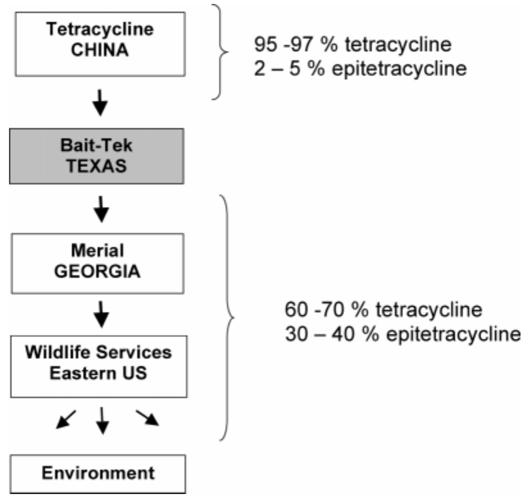

FIGURE 4. Manufacturing and distribution chain for oral rabies vaccine baits distributed by USDA/APHIS.

For the initial investigation, the purity of tetracycline (tetracycline vs. epitetracycline) in baits sampled from the end of the supply chain was compared with purity of the starting material. The initial analysis of 10 lots of the technical tetracycline indicated that the starting material used for the preparation of the bait matrix consisted of 95-98% tetracycline and 2–5% epitetracycline, a stereoisomer of tetracycline. Baits obtained from WS personnel in Ohio (USA) and West Virginia (USA) contained between 60% and 70% tetracycline and 30% and 40% epitetracycline, respectively (Fig. 4). Baits obtained from Merial and Bait-Tek were subsequently analyzed and found to have similar levels of tetracycline and epitetracycline. This indicated that the conversion of tetracycline to epitetracyc-

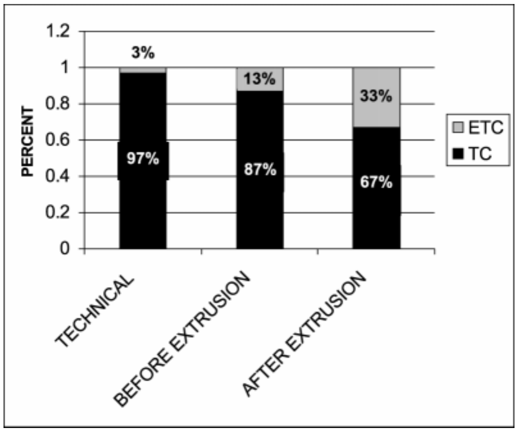

FIGURE 5. Conversion of tetracycline to epitetracycline during the manufacturing process: composition of recovered tetracyclines.

line was occurring during the manufacturing process of the fish meal polymer baits.

Bait matrix samples were obtained before and after extrusion and analyzed for tetracycline content (Fig. 5). Before extrusion, the tetracyclines in the bait matrix were comprised of 13% epitetracycline. After extrusion, the tetracycline content of the bait matrix contained 33% epitetracycline. Because the tetracycline raw material contained only 3% epitetracycline, it appeared that during the mixing process (potential exposure to light and heat), an additional 10% of the tetracycline was converted to epitetracycline. During the heat extrusion process, an additional 20% of the tetracycline was converted to epitetracycline to yield bait containing a 1:2 ratio of epitetracycline:tetracycline.

The results of the environmental chamber exposure study indicated that after 3 days of exposure, the mean epitetracycline content of the baits increased by 5%; the slope during this period indicated that the percentage of epitetracycline in the baits was increasing 1.5%/day. Following 3 more days exposure, the mean epitetracycline content had increased another 1.5%; the slope during this period indicated an epitetracycline increase of only 0.43%/day. The reduced slope of the epitetracycline content vs. time of exposure curve is likely because of the limited permeability of the baits to light; light-induced conversion of tetracycline to epitetracycline is limited to tetracycline near the surface of the baits.

To evaluate the potential for leaching losses of tetracycline from baits exposed to rain, baits were placed in a glass beaker of water for 1 hr. Analysis of the baits and the water indicated that only 2% of total tetracyclines had leached into the water. Furthermore, the epitetracycline:tetracycline ratio in the baits was unchanged by the soaking process.

To determine if the conversion of significant quantities of tetracycline to epitetracycline would impact the biomarking ability of the tetracycline containing baits, a raccoon-feeding study was conducted with tetracycline and epitetracycline. The tetracycline biomarker was detected in 88% (seven of eight) raccoons fed 150 mg tetracycline (the target content of tetracycline in one bait). In the epitetracycline (<2% tetracycline) fed group, the tetracycline biomarker was detected in only 44% of raccoons. No biomarker was detected in teeth collected from control animals, indicating that the animal feed and water did not contain tetracycline. Although it has been suggested that the ability of tetracycline to mark an animal may be influenced by age (Johnston, 2001), it appears that the age of the test animals did not have a significant affect on the results of this study. For example, ages ranged from 1 yr to 3 yr in the tetracycline-exposed animals; the only animal that was not tetracycline positive was 2-yr old. In the epitetracycline-exposed group, ages ranged from 1 yr to 5 yr. Again, a visual inspection of the fraction of animals marked in each age group (1 yr, 50%; 2 yr, 50%; 4 yr, 0%; 5 yr, 100%) strongly suggests the lack of an age-related trend with respect to tetracycline-marking ability (Table 3). Finally, the Chi-square test results indicated that age did not have a significant effect on the probability of detecting tetracycline marking in exposed animals. As all the deposits appeared to have been

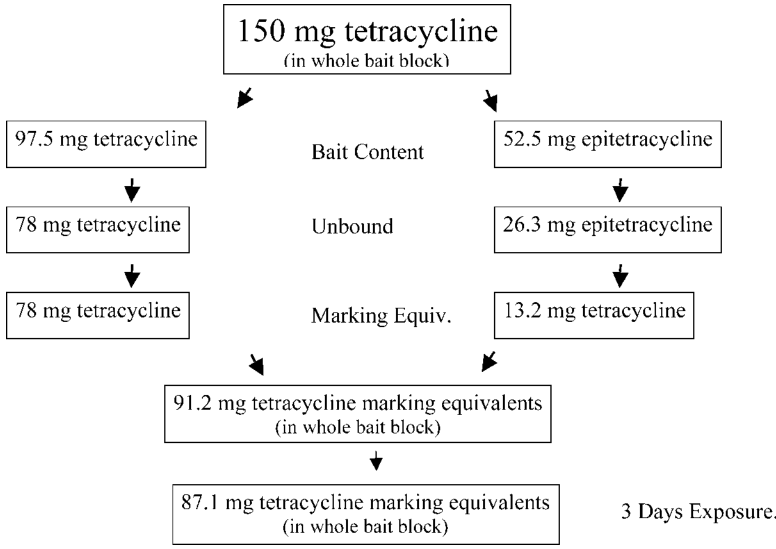

FIGURE 6. Estimated tetracycline biomarker equivalents in rabies vaccine baits.

formed during the past 12 mo, the probability that precapture exposure to tetracycline contributed to these results is eliminated. These findings indicate that as a biomarker, epitetracycline is only one-half as effective as tetracycline.

To ascertain the percentage of tetracycline trapped in the plastic polymer component of the bait, tetracycline was quantified with and without the dichloromethane solubilization step. The results of these analyses indicated that 20% of the tetracycline and 50% of the epitetracycline content of the baits was trapped in the plastic. This may be significant with respect to the biomarking potential of the baits because it is likely that polymer-encapsulated tetracyclines pass through the gastrointestinal tract and are excreted without being absorbed by the animal.

Based on these studies, the total biomarker potential, in tetracycline equivalents, was estimated for the baits (Fig. 6). The target tetracycline concentration in the baits was 150 mg. As analysis of the baits before field distribution indicated that the tetracyclines existed in a 1:2 epitetracycline:tetracycline ratio, these baits contained an average of 100-mg tetracycline and 50-mg epitetracycline. As the plastic polymer component of the bait encapsulated 20 and 50% of the tetracycline and epitetracycline, respectively, approximately 80-mg tetracycline and 25-mg epitetracycline was available for absorption from each bait. Because epitetracycline exhibited only one half the marking potential of tetracycline, the epitetracycline content had the marking potential equivalent to approximately 13-mg tetracycline. If consumed by an animal shortly after distribution, the bait contained the marking potential equivalent of 91-mg tetracycline. If this bait remained in the environment for several days, the marking potential would decrease to 87-mg tetracycline equivalents or roughly 60% of the target tetracycline concentration.

In a recent feeding study, six raccoons ranging from 0.75 yr to 5.75 yr of age were fed 0, 2, or 4 rabies baits. Mandibular and maxillary teeth were extracted from each animal and analyzed for the presence of the biomarker using UV and polarized light microscopy (Johnston and Watt, 1981). No biomarker was detected for the control animals. One tetracycline band for each bait consumed was detected for all animals except for an 8.8 kg, 5.75-yr old raccoon that had consumed 60% of the bait (Maki, 2004). This dose would be equivalent to that of a 14.5-kg raccoon that had consumed an entire bait.

These observations suggest that the conversion of tetracycline to epitetracycline decreases the marking potential of the baits and can result in baits with marginal marking potential for larger animals. This conversion may contribute to the discrepancy between rabies neutralizing antibody titers and biomarker presence observed in field-collected samples and suggests that future bait formulation studies to improve the biomarker effectiveness of the baits should be considered. The findings from this study also emphasize the importance of analyzing the biomarker content of baits rather than assuming the composition of the final product based solely on raw ingredients.

ACKNOWLEDGMENTS

The animal-handling assistance of Susan Jojola, Stacie Robinson, and the NWRC Animal Care Staff was critical to the success of this project. Brandon Schmit's contributions to multiple aspects of this study are greatly appreciated. The cooperation of Mal Smith (Bait Tek) and Joanne Maki (Merial) is greatly appreciated. This research was funded in part by the USDA/APHIS/WS national rabies program.

LITERATURE CITED

ANDERSON, R. L. 1987. Practical statistics for analytical chemists. Van Nostrand Reihnold. New York, New York. 316 pp.

ANONYMOUS. 2003. Air raid conducted on rabies in raccoons in Tennessee, Virginia. Journal of the American Veterinary Medical Association. 223: 1408–1408.

BACHMAN, P., R. N. BRAMWELL, S. J. FRASER, D. A. GILMORE, D. H. JOHNSTON, K. F. LAWSON, C. D. MACINNES, F. O. MATEJKA, H. E. MILES, M. A. PEDDE, D. R. VOIGT. 1990. Wild carnivore acceptance of baits for delivery of liquid rabies vaccine. Journal of Wildlife Diseases 26: 486–501.

CENTERS FOR DISEASE CONTROL. 1986. Rabies surveillance annual summary. Centers for Disease Control, Atlanta, Georgia. 24 pp.

COWAN, D. P., J. A. VAUGHAN, K. J. PROUT, AND W. G. CHRISTER. 1984. Markers for measuring bait consumption by the European wild rabbit. Journal of Wildlife Management 48: 1403–1409.

CRIER, J. K. 1970 Tetracyclines as a fluorescent marker in bones and teeth of rodents. Journal of Wildlife Management 34: 8290–834.

GORDON, E. R., A. T. CURNS, J. W. KREBS, C. E. RUPPRECHT, L. A. REAL, AND J. E. CHILD. 2004. Temporal dynamics of rabies in a wildlife host and the risk of cross-species transmission. Epidemiology and Infection 132: 515–524.

HANLON, C. L., D. E. HAYES, A. N. HAMIR, D. E. SNYDER, S. JENKINS, C. P. HABLE, AND C. E. RUPPRECHT. 1989. Proposed field evaluation of rabies recombinant vaccine for raccoons (*Procyon lotor*) Journal of Wildlife Diseases 25: 555–567.

HEMLICK, C. G. 1983. The epidemiology of human rabies post-exposure prophylaxis 1980-1981. Journal of the American Medical Association. 250: 1990–1996.

JOHNSTON, D. H., AND I. D. WATT. 1981. A rapid method for sectioning undercalcified carnivore teeth for aging. In World furbearer conference proceedings, August 3–11, 1980. Frostburg, Maryland, J. A. Chapman and D. Pursley (eds.). 1: 407–422.

JONES, M. E., A. T. CURNS, J. W. KREBS, AND J. E. CHILDS. 2003. Environmental and human demographic features associated with epizootic raccoon rabies in Maryland, Pennsylvania, and Virginia. Journal of Wildlife Diseases 39: 869–874.

KREBS, J. W., J. T. WHEELING, AND J. E. CHILDS. 2002. Public veterinary medicine: Public health—Rabies surveillance in the United States during 2002. Journal of the American Veterinary Medical Association. 223: 1736–1748.

KUEHN, B. M. 2003. Virginia joins USDA efforts to stop spread of rabies in raccoons. Journal of the American Veterinary Medical Association 221: 1090–1090.

LEFEBVRE, L. W., J. E. PENDERGAST, AND D. G. DECKER. 1988. Tetracyclines as fluorescent bone markers in cotton and roof rats. In Vertebrate pest control and management materials, S. A. Shumake and R. W. Bullard (eds.). ASTM STP 974, American Society for Testing and Materials, Philadelphia, Pennsylvania, pp. 134–138.

LINHART, S. B. AND J. J. KENNELLY. 1967. Fluorescent bone labeling of coyotes with dimethylchloretracycline. Journal of Wildlife Management 31: 317–321.

MAKI, J. 2004. Tetracycline biomarker analysis of rabies bait blocks. In The 6th rabies management team meeting, Atlanta, Georgia, 4 April; Centers for Disease Control.

MATSON, G. M. 2003. Workbook for cementum analyses. Matson's Laboratory, Milltown, Montana. 30 pp.

MCLEAN, R. G. 1975. Raccoon rabies. In The natural history of rabies, Vol. 2, G. M. Baer (ed.). Academic Press, New York, New York. pp. 53–77.

MILCH, R. A., D. P. RALL, AND J. E. TOBIE. 1957.

Bone localization of tetracyclines. Journal of the National Cancer Institute. 19: 87–91.
OLSON, C. A., K. D. MITCHELL, P. A. WERNER. 2000. Bait ingestion by free-ranging raccoons and nontarget species in an oral rabies vaccine field trial in Florida. Journal of Wildlife Diseases 36: 734–743.
PERRY, B. D., N. GARNER, S. R. JENKINS, K. MCCLOSKEY, D. H. JOHNSTON. 1989. A study of techniques for the distribution of oral rabies vaccine to wild raccoon populations. Journal of Wildlife Diseases 25: 206–217.
ROSATTE, R. C., AND K. F. LAWSON. 2001. Acceptance of baits for delivery of oral rabies vaccine to raccoons. Journal of Wildlife Diseases 37: 730–739.
SAVARIE, P. J., B. E. JOHNS, S. E. GADDIS. 1992. A review of chemical and particle marking agents used for studying vertebrate pests. In Proceedings of the 15th annual vertebrate pest conference, Newport Beach, California, 3-5 March, J. E. Borrecco and R. W. Marsh (eds.). University of California-Davis Press, pp. 252–257.
SLATE, D. 2003. personal communication. United States Department of Agriculture Rabies Program Manager. March 13, 2003.
TIERKEL, E. S. 1975. Canine rabies. In The natural history of rabies, Vol. 1, G. M. Baer (ed.). Academic Press, New York, New York. pp. 123–137.
WINKLER, W. G. 1986. Current status of rabies in the United States. In Rabies concepts for medical professionals, 2nd ed., D. B. Fishbein, L. A. Sawlyer, and W. G. Winklder (eds.). Merieux Institute, Miami, Florida. pp. 17–28.

Received for publication 6 August 2004.