IRB EFFICACY REVIEW

PRODUCT NAME:	ROZOL PRAIRIE DOG BAIT
PRODUCT NO.:	7173-EIA
APPLICANT:	Liphatech, Inc. Milwaukee, WI 53209
DATE COMPLETED:	2/11/09
DP NUMBER:	350015
DECISION NUMBER:	389136
DATE OF SUBMISSION:	1/23/08 (received 1/30/08, sent for review 3/3/08)
ACTIVE INGREDIENT:	Chlorophacinone
FORMULATION:	0.005% a.i. grain bait
TYPE OF PRODUCT:	Rodenticide
PURPOSE:	Product registration: new use under §3 of FIFRA
DATA MRID NUMBERS:	473336-01, 473336-02, and 473336-03
GLP CLAIMED:	Yes
TEAM REVIEWER:	Daniel B. Peacock
EFFICACY REVIEWER:	William W. Jacobs, Ph. D.
SECONDARY REVIEWER:	John Hebert, Product Manager 7 John Hebert 3/18/09

BACKGROUND

This product is a 0.005% Chlorophacinone grain bait proposed for Federal registration as a "**RESTRICTED USE PESTICIDE**" for use only

> In underground applications to control black-tailed prairie dogs (*Cynomys ludovicianus*) on rangeland and noncrop areas in the states of Arizona, Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas and Wyoming.

The proposed label stipulates that "Bait must be applied at least 6 inches down prairie dog burrows" and directs would-be uses to

> Apply bait only between October 1 and March 15 of the following year, or before spring green-up of prairie grasses, whichever occurs later.

See efficacy reviews of 7/2/04 for KS-040004 and 1/9/08 for KS-070003. Those products were registered under §24(c) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for special local needs (SLNs) in Kansas to control black-tailed prairie dogs.[1] The registration of KS-070003 was issued to supplant that of KS-040004, which was canceled on 10/14/08. SLN registrations for use of Chlorophacinone baits to control black-tailed prairie dogs have been issued in Colorado (CO-060009), Nebraska (NE-060001), Oklahoma (OK-080002), and Texas (TX-070008). In Wyoming, SLN registrations (WY-060004 and WY-070005) have been issued for Chlorophacinone bait products claimed to control "PRAIRIE DOGS (*Cynomys* spp.)".

The items routed for this efficacy review appear to be components of the original application to register this product. These items include:

- a letter dated "23 January , 2008" from Thomas Schmit, Liphatech's Manager of Regulatory Affairs, to John Hebert, Product Manager 7, Insecticide-Rodenticide Branch (IRB);
- a completed pesticide registration application form, **EPA Form 8570-1**, dated "23 January 2008" and signed by Schmit;
- an 8-page "**DATA MATRIX**" dated "23 Jan 2008" and signed by Schmit for "**Rozol Rodenticide Technical Powder**", EPA Reg. No. 7173-75;
- a "**TRANSMITTAL DOCUMENT**" dated "**23 January 2008**" and signed by Schmit;
- a black-and-white proposed label pin-punched "01•30•08"; and
- single copies two reports containing efficacy data.

A color copy of a proposed label that otherwise corresponds to the black-and-white version pin-punched "01•30•08" was added to in the efficacy review package. That item was attached to an e-mail note from Rachel Callies of Liphatech to Daniel Peacock of IRB.

The label proposed for this product would pertain to package sizes of "**1 pounds** [sic] **up to 50 lbs.**"

A copy of the ecological effects review of 7/27/06 pertaining to the Chlorophacinone SLN products NE-060001 and WY-060004 was made available to me shortly after I received the efficacy review package for 7173-EIA.

The "**DATA PACKAGE BEAN SHEET**" associated with the efficacy review package notes that the registrant is seeking to replace its existing §24(c) products claimed to control prairie dogs with 7173-EIA, which is proposed to be made available in more states than currently are covered by SLN registrations. Liphatech acknowledges as much in its application materials. According to information obtained through the website for the Smithsonian Institution's Natural History Museum, black-tailed prairie dogs occur in all 11 of the States listed on the label proposed for 7173-EIA. White-tailed prairie dogs (*C. leucurus*) and/or Gunnison prairie dogs (*C. gunnisoni*) also occur in several of the listed States.

DATA SUMMARY

Formulation

See confidential attachment to this review.

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[1] The label originally accepted for KS-040004 by the Kansas Department of Agriculture (KDA) claimed control of "**PRAIRIE DOGS (*Cynomys Sp.*)**". The label that KDA accepted for KS-070003 claims control of "**BLACK-TAILED PRAIRIE DOGS (*Cynomys ludovicianus*)**". The black-tailed prairie dog is the only prairie dog species that occurs in the wild in Kansas.

Efficacy Data

Yoder, C.A. (2008) Acute oral toxicity (LD_{50}) Chlorophacinone in black-tailed prairie dogs (*Cynomys ludovicianus*). Unpublished report, Project No. QA-1446, National Wildlife Research Center, U.S. Department of Agriculture, Fort Collins, CO, 86 pp.

MRID# 473336-01

Yoder conducted range-finding and formal LD_{50} studies using wild-caught black-tailed prairie dogs as subjects. Chlorophacinone in Propylene Glycol solutions was administered by oral gavage. For the range-finding study, 2 animals (1 male, 1 female) were dosed at each of the following levels: 0.25, 1, 2, and 4 mg Chlorophacinone per kg of body weight. For the formal LD_{50} study, 10 animals (5 males, 5 females) were given "targeted" dosages of each of the following levels: 0, 0.25, 0.6875, 1.125, 1.5625, and 2 mg/kg. The "nominal" dosages reportedly were 0.253, 0.6867, 1.127, 1.5600 and 2 mg/kg Chlorophacinone.

Yoder (2008) identifies the test material as "Rozol® Rodenticide Chlorophacinone technical" obtained from "LiphaTech, Inc.", which was reported to be 99.4% Chlorophacinone.

The prairie dogs used in this trial were trapped in Boulder County, CO. All were estimated to be one year or more in age when captured. The animals were ear-tagged and temporarily housed individually outdoors in Tomahawk live-traps. Depending on the specific model, the traps afforded the prairie dogs 2.25 or 3 ft^2 of bottom area. Prior to dosage, each prairie dog was moved indoors and placed in a cage with 4 ft^2 of floor area, with "a length of PVC pipe ... provided as a hide", and kept on a 12-hr/12-hr light/dark cycle at 60-70ºF. Subjects were weighed on days 0, 7, 14, and 21 during the test phase of the range-finding trial.

Animals were fed "grass hay or timothy hay cubes, apples, and carrots". The amounts of these feed items offered were adjusted during both studies based upon changes in subjects' weights and their apparent preferences. Yoder did not offer alfalfa cubes due to their reported high Vitamin K_1 content.[2]

Subjects "were fasted ≥ 17 hours" before dosing in the range-finding study. After gavage, the animals were checked "for signs of regurgitation or aspiration" and then returned to their cages, where they were observed 2X/day "for signs of chlorophacinone related toxicity, including pain and distress". After one animal died, the remaining animals were observed 3-4X/day. Personnel used weigh-back procedures to assess the amounts of apples and carrots that were consumed. Feed items dispersed by rodents from cages were not included in the weigh-back assessments. Corrections for moisture loss from feed were made.

The procedures followed for the formal LD_{50} trial were similar to those for the range-finding test except for the increase in the number of subjects used and the changes in dosage levels. The pre-gavage fasting interval was ≥17 hr. After dosing, subjects were observed 2X/day and then 2-3X/day after the first death was noted. Weigh-back and visual assessments of feed consumption were conducted during the formal LD_{50} trial. Adjustments to amounts of items offered were made based upon feedback from weighing subjects, which occurred on test days 0, 7, 14, and 22.

In both trials, animals found dead also were weighed; and

> Animals that were experiencing distress and appeared unlikely to recover were euthanized.

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[2] Vitamin K_1 is antidotal to anticoagulant rodenticides such as Chlorophacinone. In a "DEVIATION TO STUDY PROTOCOL" appended to her report, Yoder states that "Grass hay should more closely mimic the levels of Vitamin K1 prairie dogs are likely to be exposed to in the wild."

That approach would have shortened times to death for some subjects and might have inflated the number of animals classed as having been killed by Chlorophacinone.

Yoder (2008) reports that

> Two animals remained alive at the end of the [full LD_{50}] study despite having exhibited symptoms for approximately a week. One of these animals appeared to have been starting to recover. This underscores the need to balance obtaining scientifically valid data with animal welfare issues. During the range-finding test, three animals were euthanized compared to one animal in the LD_{50} test.

The assayed Chlorophacinone concentrations in the Polyethylene Glycol solutions used for oral gavage were close to but somewhat off (often on the high side) from the intended concentrations. If the results of these assays were available before dosing occurred in the LD_{50} test, the amounts of solutions gavaged to subjects could have been adjusted so that the targeted dosages were administered.

The fates of animal in the range-finding study are summarized in the table shown below.

DOSAGE (mg/kg)	NUMBER TREATED	NUMBER DEAD	DAYS TO DEATH
0.25	2	0	--
0.50	2	2	7, 14
1	2	1	11
2	2	2	12, 16
4	2	2	16, 18

These results were entered into SAS Institute programs for calculating LD_{50} values. A figure of 0.51 mg/kg reportedly was obtained.[3] Yoder's (2008) report does not indicate the sexes or weights of the individual subjects, nor does it note which 3 of the 7 animal deaths reported in this trial involved euthanasia.

The quickest reported time to death was one week. Four victims lasted 2 weeks or longer, and one or more of those reported deaths might have been hastened by test personnel. Although black-tailed prairie dogs appeared to be killed by a single oral administration of Chlorophacinone, their deaths were on the slow side, even for an anticoagulant.

Yoder (2008) reports that body weights decreased over time during the range-finding study. As a compensatory measure, the amount of carrots offered to the prairie dogs was increased from 95 g on day 0 to 100 g on day 7. The amount of apples offered was increased from 60 g on day 0 to 75 g on day 7 and to 100 g on day 14. Yoder ran moisture controls on some test days to assess weight loss from apples and carrots through evaporation. Consumption data are not given for individual animals.

Yoder presents consumption data for carrots and apples as mean "**g consumed/g body weight**" per day, which amounts to the percentage of body weight consumed daily. The means tended to increase or to be stable over time for the groups (0.25 and 1 mg/kg) that had one or more survivors and to decline near the times of death in groups that had no survivors. The highest

[3] The report's "**ABSTRACT**" gives a figure of 0.49 mg/kg.

daily mean reported for carrot consumption in the range-finding trial was 0.11 g/g, which works out to about to 95 g per animal per day. That figure is equal to all of the carrots offered to the group (1 mg/kg) for which 0.11 g/g consumption was reported on 4 of the first 6 post-gavage days.

Daily mean apple consumption increased over time in the groups that had survivors, as did the amount of apples made available. The reported mean daily apple consumption on day 0 was 0.06 g/g for 4 of the 5 dosage groups. The other group reportedly consumed 0.05 g/g on day 0. Based upon their reported day-0 mean body weights, the 5 groups consumed from ~54 g to >60 g of applies on day 0, which was all or nearly all of the amounts of apples that they were offered. The highest daily mean consumption figure reported for apples as any time during the range-finding study was 0.11 g/g for the survivor in the 1-mg/kg dosage group on days 20 and 21. As that animal's day-21 weight was 765 g, it would have consumed ~84 g of the 100 g of carrots made available to it then.

Yoder notes that the animals that survived the range-finding study "were hungry and ate immediately after food was placed in the cage." That observation and the apparent fact that survivors as well as victims lost weight over time suggest that subjects were fed insufficiently and perhaps inappropriately during the range-finding trial.

Yoder reports that animals that died during the range-finding test were more likely than survivors to shown overt signs of toxicosis. This information is presented in summary numbers within the text portion of the report and in bar graphs. The information is collapsed across subjects and observation days. Thus, the data do not show the time course to the expression of the various symptoms. The frequencies of adverse symptoms reportedly observed are summarized in the table shown below.

SYMPTOM	PERCENT OF OBSERVATIONS IN WHICH SEEN	
	Victims	Survivors
External Bleeding	48.2%	3.7%
Blood in Feces	19.4%	4.1%
Days without New Fecal Deposits	12.3%	<1%
Hunched Posture	15.1%	3.6%
Prostrate	4.1%	0.0%
Ocular Symptoms*	60.5%	~11%±
Cold to Touch	5.1%	0.4%
Shallow/Irregular Respiration	10%	~2%±
Unresponsive to Cage Entry	~41%±	~10%±
Did Not Move When Touched	24.0%	4.9%
In Comatose State	3.2%	0.0%

* Includes dull eyes, swollen eyes, closed or semi-closed eyes
± Figure estimated from a bar graph

Study personnel narrowed the dosage range used in the LD_{50} trial from the "1X, 2X, 4X, 8X, and 16X" scheme identified in the protocol to "1X, 2.75X, 4.5X, 6.25X, and 8X". According to an "AMENDMENT TO STUDY PROTOCOL" document appended to Yoder's (2008) report, this change was made because

Data from the range-finding test indicate the dose needs to be no higher than 2 mg/kg and no lower than 0.25 mg/kg.

Animal fates in the LD_{50} test are summarized in the table shown below.

DOSAGE (mg/kg)	NUMBER TREATED	NUMBER DEAD	DAYS TO DEATH
0	10	0	--
0.25	10	0	--
0.6875	10	0	--
1.125	10	5	10, 10, 11, 12, 12
1.5625	10	2	19, 20
2	10	6	9, 11, 13, 14, 14, 17

From these results, the SAS program calculated an LD_{50} value of 1.8 mg/kg, with the 95% confidence range being 1.35-5.29 mg/kg. The upper extreme of this range is >2½ times the highest dosage administered in LD_{50} trial. No deaths occurred before day 9 in this trial. Yoder's report does not indicate which one of the 13 victims was euthanized.

The results of the LD_{50} test call into question the decision to narrow the dosage range, which might have been influenced by premature euthanizing of subjects in the range-finding test.

Body weights decreased over the course of the LD_{50} trial, even for the 0-mg/kg (control) group. For the groups with no mortalities, mean animal weight dropped 19.4% for the control group, 17.3% for the 0.25-mg/kg group, and 20.2% for the 0.6875-mg/kg group. The 5 survivors in the 1.125-mg/kg group averaged 27.0% less in body weight than did the group's original 10 subjects on day 0. Over that same course of time, the mean animal weight dropped 11.5% for the 1.5625-mg/kg group (8 survivors) and 26.2% for the 2-mg/kg group (4 survivors). Although it is common for animals poisoned by anticoagulants to reduce food consumption and lose body weight prior to death, 2 of the experimental groups averaged a lower percent weight loss than did the control group which was gavaged with vehicle only. Consequently, something about the test circumstances was implicated in the animal's weight loss.[4]

At the start of the post-dosing period, each prairie dog was supplied, daily, 60 g of apples, 80 g of carrots, and a 25-g timothy hay cube for nourishment. The amount of apples given was raised to 80 g on day 10.

The highest mean daily "**g consumed/g body weight**" of carrots for any group in the LD_{50} test was 0.10 g/g by the 0.25-mg/kg group over the last 7 days of the trial, during which time that group's mean body weight dropped from 852.0 g to 725.0 g. From this weight range, I calculate that the daily consumption of carrots by the 0.25-mg/kg group animals was approximately 72-85 g/animal/day -- all or nearly all of the carrots that they were offered.

[4] Prairie dogs more readily consume grains during the latter half of the year than in springtime. This dietary change might be due in part to the curing of prairie grasses, but the seeds that they and other plants produce would be expected to contain more usable nutrients per unit of dry mass than would any of the items fed to captive blacktails in these Yoder's trials. The range-finding and full LD_{50} trials were conducted, respectively, in July and August of 2007.

The 0.25-mg/kg group also had the highest daily rate of consumption of apples by the end of the LD_{50} test. The mean daily "**g consumed/ g body weight**" figure for that group rose to a high of 0.10 on Day 15 and remained at that level for the remainder of the trial. As with the carrots, the 0.25-mg/lg group consumed essentially all of the apples that they were offered. On day 0, the mean rates of apple consumption were 0.5-0.7 g/g across groups. Those rates work out to ~50 g/subject/day for the control group, ~61 g/s/day for the 0.25-mg/kg group, ~56 g/s/day for the 0.6875-mg/kg and 1.5625-mg/kg groups, and ~57 g/s/day for the 2-mg/kg group.

For the LD_{50} test, Yoder (2008) reported symptoms in the manner that she did for the range-finding test (i.e., collapsed across groups, subjects, and observation days). The table below summarizes the reported symptoms.

SYMPTOM	PERCENT OF OBSERVATIONS IN WHICH SEEN	
	Victims	Survivors
External Bleeding	26.9%	8.2%
Blood in Feces	9.5%	3.6%
Days without New Fecal Deposits	8.1%	2.3%
Hunched Posture	8.1%	1.8%
Prostrate	~5%±	~1%±
Ocular Symptoms*	23.2%	~6%±
Cold to Touch	5.1%	0.4%
Shallow/Irregular Respiration	3.1%	~1%±
Unresponsive to Cage Entry	14.4%	5.2%
Did Not Move When Touched	12.8%	2.0%
In Comatose State	~3%±	~1%±

* Includes dull eyes, swollen eyes, closed or semi-closed eyes
± Figure estimated from a bar graph

Most of the subjects that exhibited external bleeding bled from only one site. One victim bled from 5 sites. No survivor showed external bleeding from more than 2 sites.

The absences of data on individual subjects, time-course data, and raw data in general greatly limit the depth of review that the Yoder (2008) report can be given and also limit the inferences that can be drawn from it. It seems clear enough that single orally administered dosages of Chlorophacinone at dosages from approximately 0.5 to 2 mg/kg of body weight will kill some but not all black-tailed prairie dogs receiving them. Times to death were slow. That deaths occurred more than 2 weeks after a single administration suggests that Chlorophacinone remains in the body and pharmacologically active for 20 days or more. Nutritional issues sufficient to cause weight loss in non-poisoned animals might have contributed to some of the observed deaths. Most victims reportedly were symptomatic prior to their deaths.

Lee, C.D. and Hyngstrom, S. E. (2007) Field efficacy and hazards of Rozol bait for controlling black-tailed prairie dogs. Unpublished report, Liphatech, Inc., Milwaukee, WI, 300 pp.

MRID# 473336-02

This report previously was assigned MRID# 472677-01 and was considered in the efficacy review of 1/9/08 for KS-070003. Those discussions were imported into and edited for this review.

Lee of Kansas State University (Manhattan) and Hyngstrom of the University of Nebraska (Lincoln) directed the research described in this report for Liphatech. Schmit served as quality assurance officer for this project. The field phase of the study began in the autumn of 2006 and concluded in the spring of 2007.

The test substance used was described as "Rozol Pocket Gopher Bait, EPA Reg. No. 7173-184" which was reported to have SLN registrations in KS and NE. Bait lots #284061 and #19906 reportedly were used in this trial. According to a "**CERTIFICATE OF ANALYSIS**" sheet signed by Melissa Zobel on 10/12/06, lot #284061 was manufactured on 10/11/06 and, on the same day, assayed at "59.13 mg/kg" (0.005913%) Chlorophacinone. A "**CERTIFICATE OF ANALYSIS**" sheet signed by Shane Nimmer on 1/4/07 indicates that lot #19906 was manufactured on 7/18/06 and was assayed as being "53.54 mg/kg" (0.005354%) Chlorophacinone on 1/4/07. Protocol information appended to the main report indicates that the researchers intended to use a single lot of bait for this project. Bait from the older lot was used after the intended lot was depleted.

The efficacy report does not include formulation sheets for the 2 bait batches. Such documents should be obtained from Liphatech and reviewed before a final decision on the §3 registration application for 7173-EIA is rendered to verify the composition of the test material used in this trial.

The stated objectives of this research project were as quoted below.

1. Determine the efficacy of Rozol Prairie Dog Bait in controlling black-tailed prairie dogs, when applied in-burrow, at the rate of ¼ cup of bait per active burrow;

2. Determine the (approximate) number of prairie dogs that are available after death to predators/scavengers on the surface of the ground;

3. Determine the amount of granules of Rozol Prairie Dog Bait that are moved to the ground surface, out of the burrows, by the normal activity of prairie dogs, predators and scavengers or prairie dogs, or by other wildlife, livestock or domestic animals;

4. Provide carcasses of black-tailed prairie dogs collected from treated areas, for tissue analysis to determine whole-body and liver concentrations of chlorophacinone residue;

5. Determine if the time of year when application is made has measurable influence on the efficacy, availability of carcasses on the surface of the ground, and/or the tissue concentrations of chlorophacinone residue.

Animals that die within burrows will be available to certain types of predators and scavengers (e.g. mustelids) that are active during winter and to such organisms as well as to snakes and burrowing owls at other times of the year.

In the course of this research, efficacy evaluations were made in October and November of 2006 – "early season application (fall)" – November and December of 2006 – "mid-season application (early winter)" – and March and April of 2007 – "late season application (late winter)". In this review, the time periods are referenced by month. Doing so more accurately places them in time than do the seasonal descriptive terms coined by the authors. Two of the bait applications for this study were made in autumn (on 10/20/06 and 12/2/06). The third application, on 3/9/07, was in winter.

Tests were run at a total of 13 sites located in Kansas and Nebraska. Within seasonal trials, study areas were geographically relatively close to one another. A monitored untreated control (check) site was included in the design for each of the seasonal trials. Two test plots were baited in October of 2006. Four plots were baited in December of 2006; and 4 more were baited in March of 2007. Information on locations, areas, and treatment groups for study sites is summarized in Table 1.

Two indices for evaluating prairie dogs activity were used before and after bait applications: visual counts and closed burrows. These methods often are employed in field efficacy trials involving prairie dogs or ground squirrels. The 2 methods were to be run sequentially according to protocol appended to the research report, but entries on raw data sheets indicate that they often overlapped in time. These methods typically are not run concurrently. Closing burrows and the human activity associated therewith might affect the number of prairie dogs visible above ground.

As it is typically performed in field efficacy trials, the visual counts method entails 3 scans conducted on each monitored plot over the same time period each day (within plots) for 3 consecutive days. The highest number of target species animals seen during the 9 scans is taken as the index for the census period. For this trial, 2 scans were taken on the morning and 2 more in the afternoon of one or two days (Table 1). The highest number of prairie dogs seen in any one scan became the index figure for that plot (Table 2). After the visual counts scans were completed, burrows were to be plugged with earth for the start of closed-burrows assessments. However, scans for the visual counts method sometimes were conducted between the times when burrows were closed and re-checked (see Table 1).

For the closed burrows method, the typical procedure is to close some or all of the seemingly active burrow openings within the census area, mark the closed burrows with flagging, and re-examine them subsequently for signs consistent with opening by targeted rodents. The usual interval between when burrows are plugged and when they are reopened is 48 hours, but re-checking after 24 hours sometimes is practiced. Lee and Hyngstrom (2007) re-checked burrows after 24 hours. According to the protocol for this study, burrows were to be considered as active only if evidence found upon re-inspection was consistent with their having been opened by prairie dogs (as opposed to a "non-target animal"). As noted in Table 3, some burrows on at least one plot apparently were judged to have been opened by "*rodents*" other than prairie dogs.

On each study plot, 100 seemingly active burrows were to be plugged and marked with turf paint. These burrows were to be on 2 transect lines of 50 burrows each. Transect lines were intended to cross and to be "approximately perpendicular" to one another. If the colony size, shape, and burrow density did not permit two crossing lines of 50 active burrows each, procedures were to be adapted to get to 50 burrows using other systematic means. In the end, the researchers decreased the number of seemingly active burrows plugged and marked to 50 (2 crossing transects of 25 burrows each) for "the smallest colonies" involved in these trials. As can be seen from Table 3, there were 3 such "smallest" colonies in the March-April trial and none in the earlier trials. All plots had two transect lines established for assessing burrow activity. "Plot Diagrams" presented in "Appendix 3" to the Lee and Hyngstrom (2007) report suggest that one or more of the transect lines used to assess efficacy extended virtually to at least one edge of many of the study plots. Additional transects were established on baited plots to assess bait availability and to search for carcasses. Transects for carcass searches extended beyond the edges of the plots.

In this study, the 2 census methods often overlapped temporally, with the total period of time elapsing from their initiation to their completion being 2 or 3 days (Table 1). Short census periods notwithstanding, the researchers used a number of interesting methods of data analysis. Some of these involved indices for grain removal and relocation which employed conversions of continuous-variable data into what essentially amounted to ranks.

Each bait application

> was made by qualified applicator, who holds the appropriate license for state where the study plots were located.

The authors state that ¼ cup of bait was deposited into each active burrow and that sufficient

> Care was taken to place the bait at least 6 inches into the burrow, and no bait was left on the surface of the ground. Any bait that was spilled above ground or placed less than 6 inches down the burrow was removed before proceeding to the next burrow. A count of the number of burrows treated was maintained and recorded by the investigator(s) making the bait application. Bait application was made by hand, or with a dispensing device mounted on an all-terrain vehicle.

The ¼-cup amounts of bait were measured in kitchen-type measuring cups. Apparently, different cups were used at different sites. Cups were not calibrated as to exact capacity but were "used in a consistent manner when placing bait in prairie dog burrows".[5] Lee and Hyngstrom (2007) state that there were "approximately 1650 grains" in each ¼ cup of bait.

According to protocol information in "Appendix D" to the protocol ("in Appendix 4" to the main report),

> Any bait dispensing device used must be calibrated to ensure that it dispenses the correct amount, with minimal variation between "doses" dispensed by the machine. The performance of the machine will be documented by calibration prior to the first use on any day, and at the conclusion of baiting at the end of the work day, following any damage, repair or adjustment made to the device, and following the transportation of the device to another site, according to the following procedure ... [not quoted here].

Calibrations of the "*Prairie Dog Feeder*" equipment used on many of the treated plots in the Lee and Hyngstrom (2007) trial ran in the ~47- to 53-g range (roughly 1.6-1.9 oz). There was close agreement in "dose" weights within runs of 10 calibration checks; but runs conducted on the same day, perhaps with different equipment, could be a few grams apart in central tendency and varied between applicators. The proposed label for 7173-EIA directs that "¼ cup (53 grams or nearly 2 ounces)" of bait be applied "at least 6 inches down active prairie dog burrows." The 53-g (~1.9 oz) amount exceeds the average amount of bait delivered in every calibration trial for the "*Prairie Dog Feeder*" and the expected weight of ¼ cup of the test bait. To maximize chances for effective treatment and to make operational use as consistent as possible with what went down holes in the Lee and Hyngstrom (2007) field trial, the label should direct use of a bit more than ¼ cup of bait per hole. Calling for a "heaping ¼ cup" might get the amount right but almost certainly would increase the likelihood of bait being spilled on the surface. Calling for "a shallow ⅓ cup of bait" might get the mass of bait used per hole closer to what went down in the field trial and reduce the incidence of spillage as well.

Based upon information supplied by the registrant, ⅓ cup of the 7173-184 product should weigh about 2.1 oz (60 g). However, Liphatech did not alter the bulk density figure claimed for 7173-184 when that product's formulation was amended in 2005. If the density figure provided by Liphatech for 7173-184 is incorrect, my calculations regarding the weights of ¼ and ⅓ cup of that bait would be inaccurate.

Bait reportedly was applied to entire prairie dog towns. Each town was treated once. These towns were 2.1-41.5 acres in area and were isolated from one another by "roadways, other

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[5] From information supplied for 7173-184, I calculate for the efficacy review of 1/9/08 for KS-070003 that ¼ cup of bait should have weighed about 1.6 oz (45.3 g). A single round of treatment at the rate of ¼ cup/burrow opening would provide more or less than that amount of bait per prairie dog depending upon whether the number of openings was greater or less than the number of live prairie dogs.

natural or artificial barriers, or large areas of land not occupied by prairie dogs." There apparently were no treated buffer zones, as such, surrounding census areas. However, areas adjacent to census plots apparently were treated at two sites (see notes to Table 1). Each town reportedly "contained at least 20 individual animals."

"Bait Availability on the Ground Surface" was assessed over the first 7 days of bait exposure. The study plan called for a transect of 50 burrows per colony to be established for this index and for inspectors to assess how much bait was found on the ground surface and how much was greater or less than 6 inches down the hole.

Following treatments, personnel reportedly engaged in systematic carcass searches within plots and extending ~100 feet beyond their borders "in all directions". Eight to 11 searches were performed on each of the treated plots. Searches

> were conducted during afternoon hours (weather permitting) to minimize the availability of carcasses to nocturnal predators/scavengers.

Such a procedure likely would not routinely be followed during operational use of a product such as 7173-EIA.[6] The label proposed for this product requires 2 post-treatment visits to baited plots. The first visit is to occur

> within 5 to 10 days after bait application, to collect and properly dispose of any bait or dead or dying prairie dogs that may have come to the surface. A second carcass search and collection must be made 14 to 21 days after bait application.

The protocol for the Lee and Hyngstrom (2007) trials called for "one recovered prairie dog carcass" from each poisoned plot to be collected and frozen for subsequent residue analyses.

The authors note that weather conditions affected when some study procedures were conducted. The period of time elapsing between bait applications and the conclusion of census activities and carcass searches varied from season to season, being 22 days for the October-November trials, 26 days for the November-December trials, and 23 days for the March-April trials.

At one site, the land owner

> Treated and covered all active burrows prior to the final plugged burrow count and visual observation.

Those activities corrupted the post-treatment activity assessments for that site. The visual counts method was used (with questionable results), but the closed-burrows method was not attempted. The copies of raw data sheets appended to the main report include the notation that, at that site,

> *Landowner plugged all burrows yesterday "so they would not move back in." He said he saw no pdogs but some digging since the rain. Used exploder on 4-1-07 am!*

Use of a burrow-exploding device on 4/1/07 preceded the afternoon visual counts survey conducted that afternoon and the morning visual counts survey conducted on 4/2/07. One prairie dog reportedly was seen on the plot on during each of the 4 scans conducted on those days

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[6] A primary objection to using Zinc Phosphide to control prairie dogs is the need to prebait colonies with untreated grain corresponding to that used in the toxic bait before the latter is applied. It seems unlikely that individuals reluctant to prebait would readily make repeated visits to baited sites to search for and remove or bury animal carcasses.

(Table 2). Thus, sequential use of the bait and the device did not eliminate prairie dog activity at that site.

The efficacy estimates obtained via the visual counts and closed-burrows methods are summarized in Tables 2 and 3, respectively. For treatment periods in which there was a decline in the relevant index on the untreated plot, the estimate of the effects of treatment on the index has been adjusted downward accordingly in those Tables.[7] On all treated plots, post-treatment activity as measured by either index was much lower than during the pretreatment census period. Where applicable, adjustments for results from check plots altered the estimates from poisoned plots very little.

Due to the temporal overlapping of the two census methods, it is possible that deliberately closing burrows would have reduced the number of prairie dogs visible above ground. In treated plots, the number of burrow openings plugged to census activity was a minority, often a small minority, of those that were treated. Therefore, the effects of human-closed burrows on the number of places from which prairie dogs could make themselves visible could have been relatively slight. It is not clear whether and to what degree the recent human presence and shoveling activities on the plots affected the willingness of blacktails to show themselves while humans were observing the plots.

Raw and adjusted post-treatment reductions in activity indices greatly exceeded the 70% (minimum) activity reduction criterion set forth in our guidelines for field efficacy studies of lethal rodenticides. The effects of overlapping of census methods on activity indices probably were small, especially where 25% or less of the burrows at the site were plugged during the visual counts scans. Therefore, it seems unlikely that the data were confounded to such an extent that actual effects of treatment that were less than 70% were made to appear to be much greater. It seems, then, that satisfactory levels of control of black-tailed prairie dogs were achieved on all treated plots, except for the Magnani site in the March-April trial where the landowner's behavior confounded all estimates of efficacy.

That such levels of control could be obtained through placing <2 oz of bait in each burrow opening seems puzzling, especially considering the body size of adult blacktails. There likely would be enough Chlorophacinone present from 1.6- to 1.9-oz placements to control all prairie dogs present if they shared the bait relatively equally and did not consume it all at one feeding. Anticoagulants are slow-acting compounds that do not affect rodents' behavior and food consumption very much (except perhaps for their foraging strategies) over the first 2-4 days after feeding begins, after which time animals that have ingested sufficient amounts of the poison weaken and die.

The minimum single-point placement amount for controlling commensal rats with anticoagulant baits is set at 4-oz (or the rough equivalent in numbers of placepacks or bait blocks). Although the density of placements in prairie dog burrow openings may exceed that realized in the 15- to 30-foot spacing of placements when commensal rodents are targeted, adult blacktails are $\geq$2 times the size of adult Norway rats. It may be that blacktails are extremely sensitive to Chlorophacinone at all times or over winter, when stresses from lower temperatures might enhance the effects of anticoagulants.

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[7] Lee and Hyngstrom (2008) adjusted control estimates in instances when there was a "post-treatment" increase in activity on the relevant untreated plot. Such adjustments are based on assumptions that there would have been similar increases on the poisoned plots but for the use of the bait. The traditional and more conservative approach is to regard negative "post-treatment" effects on check plots as suggesting that other factors (e.g., seasonal effects, "natural" mortality at a time of year with no reproductive recruitment, etc.) might have acted to reduce activity independent of use of the bait. When activity indices increase on check plots after the time of treatment, the conservative assumption is that the effects of any negative influences other than baiting on the activity of the targeted species are unlikely to have been significant. Consequently, activity assessments from treated plots are not adjusted.

Lee and Hyngstrom (2007) report that "Bait was available in and around burrows on all colonies up to 7 days after treatment", with "all colonies" presumably meaning "all treated colonies". As would be expected, the amount of bait visible within and near burrows declined over time following application. The green dye on the bait should have facilitated its detection.

Researchers assessed all treated sites for amounts of visible bait and the depths at which it was observed varied among treated colonies (Tables 4a, 4b, 4c, 5a, 5b, and 5c). Some bait was observed on the surface on at least one occasion following application during each of the baiting periods, although surface bait reportedly was seen on only one day and in only one of the four colonies that were treated in March of 2007. On 2 of the 10 towns that were baited, across treatment seasons, there reportedly was no bait observed 7 days following application in or around any of the 50 burrow openings that were evaluated for bait availability. For the other 8 towns, the percent of burrows with visible bait a week after treatment ranged from 2% to 24%. The 2 towns with the highest amount of burrows showing some evidence of bait after a week were the Kansas towns involved in the December baiting. In those 2 towns and in all colonies involved in the March baiting, the most common depth at which bait reportedly was observed was 0-6 inches.[8]

Bait observed ≥6 inches down a hole would be at a depth consistent with proper application, whereas bait closer to the surface or on it either would have been applied inaccurately or moved upward by non-human agents such as prairie dogs, other animals, or air currents. Lee and Hyngstrom (2007) used a rating system for amounts of bait found on the surface and at depths of 0-6 inches or >6 inches. Numbers in the range of <25 grains observed at specified depth ranges were assigned the "Grain Index" number of "13" (i.e., the median of the numbers between 0 and 25). Similarly, counts or estimates of 25-100 grains were given an index of "63". Observations of >100 grains were assigned the seemingly arbitrary score of "113" (perhaps to maintain an interval of 50 between successive scores). Lee and Hyngstrom calculated overall Grain Index figures by summing the occurrences of results in each of these 3 ranges.

Tables 5a, 5b, and 5c to this review present the occurrences of bait observations at the depths and amount ranges that are indicated on raw data sheets appended to the Lee and Hyngstrom (2007) report. Across treated towns, reported observations of bait on the surface were most commonly in the <25 grains range, with the only instances of more than 25 grains being observed on the surface occurring within 3 days of application. Apparently, one or more types of agents tended to relocate and/or consume grains that had gotten to the surface. Numbers of grains ≥25 were more commonly reported where bait was seen within burrows at depths of 0-6 inches or >6 inches. At such depths, the presence of bait and the numbers of grains observed declined over time, again suggesting relocation and/or consumption.

The two different methods of putting bait into burrows – hand (measuring cup) and mechanical – used in this study might have affected the tendency for bait to be seen on the surface and/or at depths <6 inches. Table 6 sorts the 10 treated sites according to whether they were treated by hand only, by "*Prairie Dog Feeder*", or by a combination of those 2 methods. Only the observations made one day after treatment are considered in Table 6 as the Day-1 observations were the recorded ones made closest to the time of treatment. Table 6 shows clearly that, regardless of the method(s) used to treat burrows, only a small percent (0-6%) of holes had visible bait on the ground surface surrounding them. That 30-72% of holes had no bait visible in or around them a day after treatment seems to mean that some agent(s) consumed, removed, or otherwise concealed the particles. The proportion of holes with no bait seen on Day 1 in positions inconsistent with the requirement to place bait at depths ≥6 inches, varied from 50% to

[8] The raw data sheets indicate only one depth at which bait was observed for each burrow for each day of observation. That circumstance probably means that the observations were scored according to the shallowest depth at which bait was observed and, therefore, that a report of bait being observed on the surface probably did not mean that there was no bait observed in the hole.

98% among assessed burrows on the 10 poisoned plots. The 3 plots with the fewest observed holes showing bait less than 6 inches down them were among the 4 that were treated strictly by hand. However, the fourth plot (Wiese West) that was hand-baited had visible bait 0-6 inches deep, one day after treatment, in nearly half of the observed burrows.

First-generation anticoagulants such Chlorophacinone tend to be much more toxic on a mg-poison/kg-body-weight basis when consumed in small amounts over several days rather than when the same amount is eaten on one day (e.g., Ashton, *et al*, 1987). That there was some bait remaining over several days of treatment would have made it possible for the bait to poison prairie dogs more efficiently than might have been the case had all bait been eaten on the first day. However, what happened to the baits that were not observed can only be inferred from other evidence. From the post-treatment declines in indices prairie to dog activity, including fewer live blacktails being seen, it seems highly likely that much of the missing bait was consumed by the target species.

Lee and Hyngstrom (2007) report that the 10 animal carcasses found above ground across the 10 poisoned towns included 9 prairie dogs. The earliest carcass findings were made 10 days post application. The last was made 25 days after bait was applied. Eight of the 10 carcasses were "completely intact", but the other 2 "had been scavenged." Prairie dog carcasses were found at a rate of 1 per 14 acres searched (0.07 carcasses/acre). Results of residue analyses of usable carcasses are reported in the Primus (2007) paper discussed below. The authors also report having seen "5 impaired prairie dogs" $\geq$10 days after bait applications.

A dead eastern cottontail rabbit (*Sylvilagus cuniculus*) also was found following treatment.

Lee and Hyngstrom (2007) report having observed the following types of vertebrate organisms "in and around the perimeter of all sites" that might have shown interest in consuming the grain bait: meadowlarks (*Sturnella* spp.), horned larks (*Eremophila alpestris*), mourning doves (*Zenaida macroura*), rock doves (a.k.a. "pigeons", *Columba livia*), and eastern cottontails. Also observed at study sites were the following carnivorous types: killdeer (*Charadrius vociferus*), great blue herons (*Ardea herodias*), red-tailed hawks (*Buteo jamaciensis*), rough-legged hawks (*Buteo lagopus*), northern harriers (*Circus cyaneus*), and coyotes (*Canis latrans*). Except possibly for killdeer, all of these species would be expected to show some interest in hunting live prairie dogs and/or in feeding upon their carcasses.

Attempts at monitoring the effects of Chlorophacinone baiting on nontarget species in this study apparently were limited to the aforementioned carcass searches. Although those searches were numerous and covered several weeks following bait application, the efforts reportedly did not extend more than "about 100 feet in all directions" beyond the perimeters of the treated towns. Anticoagulants kill very slowly. Therefore, it seems reasonably likely that wide-ranging species such as volant birds and coyotes could have been well off site if and when they succumbed to primary and/or secondary exposure to Chlorophacinone.

Pretreatment capture and radio-equipping of nontarget species really is needed to determine reliably whether and where specific individual animals expire following treatment. Necropsies and residue analyses can be performed on carcasses to assess whether exposure to the anticoagulant occurred and, perhaps, whether the anticoagulant was the likely cause of death. However, if Chlorophacinone were being used by others within the study area, its implication in the deaths of any nontarget animal would not necessarily mean that the exposure resulted from any specific use or use pattern. The KS-040004 product, for one, likely would have been available in the vicinities of the Kansas sites involved in this field trial. One or more anticoagulant products likely would have been available for use near the Nebraska sites (e.g., NE-060001).

As noted above, 0-6% of observed burrows had bait exposed on the ground surface one day after treatment, and there was some evidence of surface bait visible as late as a week post-treatment (Tables 4a, 4b, 4c, 5a, 5b, 5c, and 6). Bait less than 6 inches deep, including surface bait, was observed at all treated sites on 5-7 of the days covering the first week following bait application. Bait less than 6 inches deep could be reached by many types of animals, including livestock.

In light of such information, the proposed label's 3-day post-treatment grazing restriction seems inadequate. The incidence of surface bait may seem relatively low on a percent basis, but it must be remembered that hundreds or thousands of holes variously were baited on the poisoned plots involved in the Lee and Hyngstrom (2007) study. If the 6% incidence of surface bait one day following application reported at the Hogan site held for all 3088 burrows treated there, there would have been surface bait at ~185 burrows in that prairie dog town one day after treatment and at about ⅓ that many on 4 of the 6 subsequently monitored days. If the incidence of surface bait and/or bait <6 inches deep at all of the 1787 treated holes at the Ryan South occurred at the same rate as was observed at the 50 burrows sampled, ~786 (44%) of those holes would have had bait potentially accessible to livestock a day after treatment. Three days after treatment, when the label authorizes grazing, the 50% proportion of holes with bait at depths of <6 inches would have meant that ~894 holes might have had livestock-accessible bait. A week after treatment, ~286 holes on Ryan South might still have had livestock-accessible bait.

Post-treatment monitoring of livestock-available bait presence was not continued until an asymptote was approached, although incidences were much lower a week following treatment than they were one day after application. It seems clear enough that 3 days or a week of post-treatment grazing restrictions would not be sufficient to assure the public of safe beef (or milk, should dairy cattle be grazed on treated sites). Residue chemistry issues aside, looking at bait presence alone suggests that at least a one-month restriction should be imposed, to err on the side of safety (presumably).

Primus, T.M. (2007) Determination of Chlorophacinone residues in prairie dog whole body and liver tissues. Unpublished report, Project No. QA-1405, National Wildlife Research Center, U.S. Department of Agriculture, Fort Collins, CO, 58 pp.

MRID# 473336-03

Primus (2007) reports on 2 series of assays of carcasses for Chlorophacinone residues, the second of which pertains to animals collected in the Lee and Hyngstrom (2007) study.

The first series involved a sample of 12 prairie dog carcasses. Of those, 8 were judged to be "in acceptable condition for analysis". The other 4

> were desiccated or eviscerated to the point that insufficient tissue was available or unacceptable for analysis.

At least 7 of the 8 assayed carcasses apparently were collected from a site treated with Chlorophacinone on 3/14/06. The other carcass was of an animal "Found Dead East Pasture 3/30/06". The assays were conducted on 9/6/06. The laboratory report on their results is dated "10/13/06".

In whole-body assays of the carcasses collected in 2006, Chlorophacinone was detected in each animal. Concentrations ranged from 0.849 to 2.24 ppm (limit of detection = 0.054 ppm). All liver tissue samples from those animals also tested positive for Chlorophacinone (3.28-8.31 ppm, limit of detection = 0.035 ppm). Combining residue results with adjustments for the livers' being 2.6-4.8% of total carcass weight led to calculated total carcass residue levels of 1.11-2.37 ppm.

The second laboratory report pertained to carcasses of 9 prairie dogs and 1 cottontail rabbit. Those carcasses were assayed during May of 2007. The sites and dates of collection reported for these carcasses are consistent with their having have been those collected during the Lee and Hyngstrom (2007) trials. One of the prairie dogs carcasses "was desiccated and eviscerated" such that it could not be assayed.

The 8 prairie dog carcasses assayed had whole-body residues of 0.090-1.25 ppm Chlorophacinone (limit of detection = 0.083 ppm). Liver residues in these same animals were 0.524-4.93 ppm Chlorophacinone (limit of detection = 0065 ppm). Calculated total carcass loads for these animals were 0.113-1.35 ppm.

Chlorophacinone residues in the cottontail were 0.094 ppm for whole-body and 0.448 ppm in liver for a calculated total carcass load of 0.107 ppm.

These data are consistent with the animals' having been exposed to and poisoned by Chlorophacinone. That the residues in liver were higher than in whole-body-minus-liver is consistent with findings previously reported for anticoagulant rodenticides in various species. The 100% incidence of residues among the tissues assayed indicates that predators and scavengers feeding on carcasses available due to use of Chlorophacinone bait would be exposed secondarily to the anticoagulant.

LABEL

From the standpoint of efficacy, the label proposed for 7173-EIA needs only a few changes.

The proposed "**Use restrictions:**" subsection of the "**DIRECTIONS FOR USE**" would limit use of the product to below-ground applications to control black-tailed prairie dogs in 10 states (see quoted text at the beginning of the BACKGROUND section of this review). The bait is to be applied "at least 6 inches down prairie dog burrows". The application season is to be from 10/1 of one year until 3/15 of the next "or before spring green-up of prairie grasses, whichever occurs later." As I understand them, the seasonal limitations on use of anticoagulant baits for prairie dog control are intended to protect some types of migratory raptorial birds. The migration patterns of such species might be affected by weather but probably would not be affected directly by "spring green-up". Bait acceptance may be reduced by "spring green-up", however, due to prairie dogs' preference for new- and renewed-growth vegetation. Prairie dogs' willingness to accept grain-based bait increases when the grass cures (yellows) in the late spring or in summer, well before October 1.

The "**Application:**" paragraph calls for "1/4 cup (53 grams or nearly 2 ounces)" of bait to be used per treated burrow and emphasizes the sentence "**Make sure no bait is left on the soil surface at the time of application.**" In light of the findings on bait depth reported by Lee and Hyngstrom (2007), the label should make it clearer than it does now what is meant by "at least 6 inches". As prairie dogs and other animals that occur in prairie dog towns seem to occasionally move bait to the surface or to locations within burrows that are <6 inches deep, it might be difficult to enforce against marginal misuse (i.e., occasional spillage of bait on surface or not getting the entire placement to the required depth). Whole 2-oz placements made on the surface or just inside burrow openings likely would be conspicuous due to the amount of dyed bait involved, especially if sites were inspected shortly after treatment.

If carcasses are to be buried on-site, they must be placed "in holes dug at least 18 inches deep, or in inactive burrows." Although burying carcasses is to include "covering and packing the hole or burrow with soil", those measures seem unlikely to thwart all semifossorial predators and scavengers (e.g., badgers). The expression "inactive burrows" should be expanded so that it is clear that it means burrows apparently not being used by prairie dogs or any other animals that are potentially vulnerable to secondary poisoning by Chlorophacinone.

CONCLUSIONS

1. The results of the acute oral toxicity study reported by Yoder (2008; MRID No. 473336-01) suggest that the acute oral LD of Chlorophacinone for black-tailed prairie dogs is 1.8 mg/kg of body weight, with a 95% confidence interval 1.36-5.44 mg/kg. These data might overstate

the animal's sensitivity to the anticoagulant somewhat as the test facility had difficulty maintaining the control-group subjects at their initial body weights. The possibly premature euthanizing of 3 of the 7 reported deaths in the range-finding study may have led to the use of too narrow a dosage range in the LD_{50} study.

2. The efficacy report by Lee and Hyngstrom (2007; MRID No. 473336-02) suggests that single applications of ¼ cup of bait effectively controlled black-tailed prairie dogs under the conditions of use. The census methods involved in the study overlapped in time and were conducted for shorter periods of time than is typical for field efficacy trials of rodenticides on farm and rangelands. However, the trials were adequate to support the fundamental label claim.

   Information on equipment calibration suggests that the amounts of bait dispensed by the "*Prairie Dog Feeders*" usually were 47-52+ grams but seldom reached "53 g or nearly 2 ounces" per burrow. Such amounts would seem to exceed the weight (1.6 oz) of a level ¼ cup of bait, if previously reported data on product density are accurate.

3. Residue data reported by Primus (2007; MRID No. 473336-03) indicate that all 8 black-tailed prairie dog carcasses and a cottontail rabbit carcass collected during the Lee and Hyngstrom (2007) project tested positive for Chlorophacinone in assays of liver tissue and in whole-body (minus liver). Similar results were obtained with 8 black-tailed prairie dog carcasses collected during an earlier project.

4. The comments listed below pertain to the "**DIRECTIONS FOR USE**" section of the "(02308)" proposed label for 7173-EIA.

   a. Change the second sentence of the "**Use Restrictions:**" paragraph so that it reads:

      Bait must be applied at least 6 inches down prairie dog burrows (measured from the farthest back portion of the burrow opening).

   b. Retain the proposed per-burrow application amount in the "**Application:**" paragraph if the weight of a level ¼ cup of formulated bait averages 53 g. If not, adjust the gram- and ounce-equivalents on the label to be consistent with a level ¼ cup of this product. Weigh at least 10 level ¼ cups of bait to make the weight determination. (Lee and Hyngstrom (2007) report having used volume measures to determine how much bait to use.)

   c. In the fifth (next-to-last) sentence of the "**Follow-up:**" paragraph, change "inactive burrows" to

      inactive burrows (no longer being used by prairie dogs and not used by other species).

REFERENCE

Ashton, A.D., Jackson, W.B., and Peters, H. (1987) Comparative evaluations of LD_{50} values for various anticoagulant rodenticides. In: Richards, C.G.L. and Ku, T.Y. (eds.) Control of Mammal Pests. Taylor & Francis, London, New York, Philadelphia, 187-197.

Table 1. Sites and schedules used in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02).

Site Name	General Location	Acreage	Treatment	Pretreatment Visual Counts Date(s)[AM-PM]	Post-treatment Visual Counts Date(s) [AM-PM]	Pretreatment Closed-Burrows Dates	Post-treatment Closed-Burrows Dates	Date Baited	Burrows Treated	Bait Buckets Used
TNC Control	Great Bend, KS	23.3	None	10/19/06	11/10-11/06	10/19-20/06	11/10-11/06	-	-	-
Sallee	Great Bend, KS	30.7	Rozol	10/19-18/06	11/10-9/06	10/18-19-06	11/9-10/06	10/20/06	2680	10
Hogan	Great Bend, KS	41.5	Rozol	10/19-18/06	11/10-9/06	10/18-19/06	11/9-10/06	10/20/06	3088	12.66
Ryan Control	Atwood, KS	3.8	None	12/1;11/30/06	12/27-28/06	11/30-12/1/06	12/27-28/06	-	-	-
Ryan South	Atwood, KS	24.4	Rozol	12/1;11/30/06	12/27/06	11/30-12/1/06	12/27-28/06	12/2/06	1787	?
Ryan Cemetery	Atwood, KS	14.5	Rozol	12/1;11/30/06	12/27/06	11/30-12/1/06	12/27-28/06	12/2/06	1503	?
NE East Lashley	Trenton, NE	3.8	Rozol	12/1;11/30/06	12/27/06	12/1-2/06	12/27-28/06	12/2/06	337	?
NE West Faiman	Trenton, NE	8.0	Rozol	12/1;11/30/06	12/27/06	12/1-2/6	12/27-28/06	12/2-3/06	1621	?
Josh Control	Benkleman, NE	22.2	None	3/9;3/8/07	4/2;4/1/07	3/8-8/07!	4/1-2/07	-	-	-
Sowers	Benkleman, NE	4.8	Rozol	3/8-9/07	4/2;4/1/07	3/8-9/07	4/1-2/07	3/9/07	322	?
Magnani	Benkleman, NE	3.3	Rozol	3/8/07 [no PM]	4/2;4/1/07*	3/8-9/07	none*	3/9/07	101	?
Wiese West	Benkleman, NE	2.1	Rozol	3/8/07	4/2;4/1/07	3/8-9/07	4/1-2/07	3/9/07	174	?
Wiese East	Benkleman, NE	10.6	Rozol	3/8-9/07	4/2;4/1/07	3/8-9/07	4/1-2/07	3/9/07	435**	?

Note 1: A bait application tool called "Prairie Dog Feeder" reportedly was used for all treatments at the Ryan Cemetery, Sowers, and Magnani sites. The same type of equipment was used for some of the burrow treatments at Ryan South, West Faiman, and Wiese East. All applications at Sallee, Hogan, East Lashley, and Wiese West were by hand only (no mechanical equipment used). Calibrations of "Prairie Dog Feeder" equipment nearly always put "dose" amounts between 47 and 53 g (roughly 1.6-1.9 oz) with much greater consistency within calibration checks then between them.

Note 2: At the West Faiman site, 569 additional burrows were treated in an "Area east of draw not in trial but adjacent so we treated them".

Note 3: At the Sowers site, the "66 holes on west" noted on the relevant raw data sheet as having been treated were not included in the sum total of treated burrows reported by Lee and Hyngstrom (2007) in their "Table 2". Presumably, these burrows were outside of the census area for the Sowers site.

*Post-treatment visual counts surveys were done but closed burrows were not because "landowner blew up burrows this am." Landowner reportedly "'plugged all burrows" on 3/31/07 "so they would not move back in" and then "used an exploder 4-1-07 am!"

**Burrow treatments included "297 mechanical" and "138 hand".

Table 2. Visual counts data in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02).

Site Name	Treatment	Pretreat. Visual Count 1st AM	Pretreat. Visual Count 2nd AM	Pretreat. Visual Count 1st PM	Pretreat. Visual Count 2nd PM	Mean of All Four Scan Counts	Highest Pretreat. Count	Post-treat. Visual Count 1st AM	Post-treat. Visual Count 2nd AM	Post-treat. Visual Count 1st PM	Post-treat. Visual Count 2nd PM	Mean of All Four Post-treat. Counts	Highest Post-treat. Count	% Change Pre-Post-Treatment (hi-count)	Adjusted % Change
TNC Control	None	31	40	29	31	32.75	40	36	35	24	26	30.25	36	-10.0%	--
Saltee	Rozol	63	68	42	47	55.00	68	1	0	1	2	1.00	2	-97.1%	-96.7%
Hogan	Rozol	52	57	56	71	59.00	71	2	2	4	6	3.50	6	-91.5%	-90.6%
Ryan Control	None	1	1	3	4	2.25	4	2	2	5	6	3.75	6	50.0%	--
Ryan South	Rozol	12	12	4	5	8.25	12	0	0	2	2	1.00	2	-83.3%	--
Ryan Cemetery	Rozol	4	5	11	12	8.00	12	0	0	0	0	0.00	0	-100.0%	--
NE East Lashley	Rozol	6	5	9	10	7.50	10	0	0	0	0	0.00	0	-100.0%	--
NE West Faiman	Rozol	18	18	29	31	24.00	31	0	0	0	0	0.00	0	-100.0%	--
Josh Control	None	19	21	11	12	15.75	21	34	35	31	33	33.25	35	66.7%	--
Sowers	Rozol	11	11	14	16	13.00	16	2	2	1	1	1.50	2	-87.5%	--
Magnani	Rozol	7	7	--	--	3.50	7	1	1	1	1	1.00	1	-85.7%	--*
Wiese West	Rozol	9	8	8	7	8.00	9	0	0	0	0	0.00	0	-100.0%	--
Wiese East	Rozol	9	10	14	15	12.00	15	0	0	0	0	0.00	0	-100.0%	--

*Post-treatment visual counts surveys were done but closed burrows were not because "landowner blew up burrows this am." Landowner reportedly "plugged plugged all burrows" on 3/31/07 "so they would not move back in" and then "used an exploder 4-1-07 am!"

Table 3. Closed-burrow data in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02).

Site Name	Acreage	Treatment	Burrows Baited	# Burrows Closed for Pretreat. Census	% Baited Burrows Closed for Pretreat. Census	# Census Burrows Closed for Pretreat. Census Transect 1	# Census Burrows Active during Pretreat. Census Transect 1	# Census Burrows Closed for Pretreat. Census Transect 2	# Census Burrows Active during Pretreat. Census Transect 2	Total # Census Burrows Active during Pretreat. Census	% Census Burrows Active during Pretreat. Census	# Burrows Closed for Post-treat. Census	# Census Burrows Closed for Post-treat. Census Transect 1	# Census Burrows Active during Post-treat. Census Transect 1	# Census Burrows Closed for Post-treat. Census Transect 2	# Census Burrows Active during Post-treat Census Transect 2	Total # Census Burrows Active during Post-treat. Census	% Census Burrows Active during Post-treat. Census	% Change in Burrow Activity frrom Pretreat.	Adjusted % Change In Burrow Activity frrom Pretreat.
TNC Control	23.3	None	-	100	-	50	18	50	19	37	37.0%	100	50	9	50	19	28	28.0%	-24.3%	-
Sallee	30.7	Rozol	2680	100	3.7%	50	18	50	27	45	45.0%	100	50	3	50	1	4	4.0%	-91.1%	-88.3%
Hogan	41.5	Rozol	3088	100	3.2%	50	16	50	22	38	38.0%	100	50	2	50	1	3	3.0%	-92.1%	-89.6%
Ryan Control	3.8	None	-	100	-	50	5	50	7	12	12.0%	100	50	4	50	6	10	10.0%	-16.7%	-
Ryan South**	24.4	Rozol	1787	100	5.6%	50	22	50	33	55	55.0%	100	50	0	50	0	0	0.0%	-100.0%	-100.0%
Ryan Cemetery**	14.5	Rozol	1503	100	6.7%	50	28	50	32	60	60.0%	100	50	0	50	0	0	0.0%	-100.0%	-100.0%
NE East Lashley	3.8	Rozol	337	100	29.7%	50	18	50	12	30	30.0%	100	50	1	50	0	1	1.0%	-96.7%	-96.0%
NE West Faiman**	8.0	Rozol	1621	100	6.2%	50	13	50	12	25	25.0%	100	50	0	50	0	0	0.0%	-100.0%	-100.0%
Josh Control	22.2	None	-	100	-	50	14	50	14	28	28.0%	100	50	13	50	15	28	28.0%	0.0%	20.0%
Sowers**	4.8	Rozol	322	100	31.1%	50	9	50	8	17	17.0%	100	50	1	50	0	1	1.0%	-94.1%	-94.1
Magnani**	3.3	Rozol	101	50	49.5%	25	4	25	8	12	24.0%	-	-	-	-	-	-	-	-	-
Wiese West	2.1	Rozol	174	50	28.7%	25	9	25	10	19	38.0%	50	25	0	25	0	0	0.0%	-100.0%	-100.0
Wiese East**	10.6	Rozol	435	50	11.5%	25	9	25	11	20	40.0%	50	25	3	25	0	3	6.0%	-85.0%	-85.0%

*Post-treatment visual counts surveys were done but closed burrows were not because "landowner blew up burrows this am." Landowner reportedly "plugged plugged all burrows" on 3/31/07 "so they would not move back in" and then "used an exploder 4-1-07 am!"

**Burrow treatments included "297 mechanical" and "138 hand" on the Wiese East plot, some mecanical and some by hand on Ryan South and West Fairman, and all mechanical on Ryan Cemetery, Sowers, and Magnani.

Note: In the pretreatment census in transect 1 for the Hogan plot, 11 additional burrows were reportedly "opened by rodents", apparently as opposed to "p dogs".

Table 4a. Visible bait post-treatment in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02), October, 2006, baiting.

Site Name	Acreage	Treatment	Burrows Baited	# Burrows Used to Assess Availability	# Days after Baiting	# Holes with Visible Bait	% Holes with Visible Bait	# Holes with Bait at/on Surface	# Holes with Bait 0-6" below Surface	# Holes with Bait >6" below Surface	# Holes with no Visible Bait
Sallee	30.7	Rozol	2680	50	1	24	48.0%	1	0	23	26
				50	2	4	8.0%	0	0	4	46
				50	3	2	4.0%	0	0	2	48
				50	4	3	6.0%	1	0	2	47
				50	5	2	4.0%	0	1	1	48
				50	6	3	6.0%	2	0	1	47
				50	7	1	2.0%	1	0	0	49
Hogan	41.5	Rozol	3088	50	1	14	28.0%	3	3	8	36
				50	2	10	20.0%	1	5	4	40
				50	3	4	8.0%	1	0	3	46
				50	4	3	6.0%	1	0	2	47
				50	5	2	4.0%	0	0	2	48
				50	6	2	4.0%	1	0	1	48
				50	7	0	0.0%	0	0	0	50

Table 4b. Visible bait post-treatment in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02), December, 2006, baiting.

Site Name	Acreage	Treatment	Burrows Baited	# Burrows Used to Assess Availability	# Days after Baiting	# Holes with Visible Bait	% Holes with Visible Bait	# Holes with Bait at/on Surface	# Holes with Bait 0-6" below Surface	# Holes with Bait >6" below Surface	# Holes with no Visible Bait
Ryan South	24.4	Rozol	1787	50	1	34	68.0%	2	20	12	16
				50	2	31	62.0%	2	22	7	19
				50	3	29	58.0%	2	23	4	21
				50	4	20	40.0%	0	17	3	30
				50	5	18	36.0%	0	15	3	32
				50	6	12	24.0%	0	8	4	38
				50	7	10	20.0%	0	8	2	40
Ryan Cemetery	14.5	Rozol	1503	50	1	32	64.0%	2	22	8	18
				50	2	26	52.0%	1	19	6	24
				50	3	21	42.0%	0	18	3	29
				50	4	16	32.0%	1	11	4	34
				50	5	15	30.0%	0	11	4	35
				50	6	13	26.0%	0	11	2	37
				50	7	12	24.0%	0	10	2	38
NE East Lashley	3.8	Rozol	337	50	1	30	60.0%	1	2	27	20
				50	2	12	24.0%	0	4	8	38
				50	3	5	10.0%	0	1	4	45
				50	4	3	6.0%	0	0	3	47
				50	5	3	6.0%	0	0	3	47
				50	6	2	4.0%	0	1	1	48
				50	7	2	4.0%	0	1	1	48
NE West Faiman	8.0	Rozol	1621	50	1	27	54.0%	2	12	13	23
				50	2	27	54.0%	2	14	11	23
				50	3	12	24.0%	0	11	1	38
				50	4	5	10.0%	0	5	0	45
				50	5	4	8.0%	0	3	1	46
				50	6	4	8.0%	0	4	0	46
				50	7	3	6.0%	0	3	0	47

Table 4c. Visible bait post-treatment in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02), March, 2007, baiting.

Site Name	Acreage	Treatment	Burrows Baited	# Burrows Used to Assess Availability	# Days after Baiting	# Holes with Visible Bait	% Holes with Visible Bait	# Holes with Bait at/on Surface	# Holes with Bait 0-6" below Surface	# Holes with Bait >6" below Surface	# Holes with no Visible Bait
Sowers	4.8	Rozol	322	50	1	18	36.0%	0	17	1	32
				50	2	14	28.0%	1	12	1	36
				50	3	1	2.0%	0	1	0	49
				50	4	1	2.0%	0	1	0	49
				50	5	1	2.0%	0	1	0	49
				50	6	1	2.0%	0	1	0	49
				50	7	0	0.0%	0	0	0	50
Magnani	3.3	Rozol	101	50	1	31	62.0%	0	25	6	19
				50	2	27	54.0%	0	22	5	23
				50	3	14	28.0%	0	13	1	36
				50	4	10	20.0%	0	9	1	40
				50	5	7	14.0%	0	7	0	43
				50	6	5	10.0%	0	5	0	45
Wiese West	2.1	Rozol	174	50	1	35	70.0%	0	24	11	15
				50	2	31	62.0%	0	20	11	19
				50	3	31	62.0%	0	20	11	19
				50	4	10	20.0%	0	7	3	40
				50	5	5	10.0%	0	5	0	45
				50	6	2	4.0%	0	2	0	48
				50	7	2	4.0%	0	2	0	48
Wiese East	10.6	Rozol	435	50	1	25	50.0%	0	20	5	25
				50	2	18	36.0%	0	15	3	22
				47*	3	7	14.9%	0	6	1	40
				50	4	5	10.0%	0	3	2	45
				47**	5	1	2.1%	0	0	1	46
				50	6	3	6.0%	0	1	2	47
				50	7	2	4.0%	0	1	1	48

*On this plot on this day, one burrow was not scored for visible bait at all, while two others were scored as having visible bait present but without indicating the depth at which bait was visible.

**On this plot on this day, three burrows were scored as having visible bait; but the depth at which the bait was seen was not indicated.

Table 5a. Amounts of visible bait post-treatment at various depths in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02), October, 2006, baiting.

Site Name	Acreage	Treatment	Burrows Baited	# Burrows Used to Assess Availability	# Days after Baiting	# Holes with Visible Bait	# Holes with no Visible Bait	## of Grains on Surface			## of Grains 0-6" below Surface			## of Grains >6" below Surface		
								<25	25-100	>100	<25	25-100	>100	<25	25-100	>100
Sallee	30.7	Rozol	2680	50	1	24	26	1						11	6	6
				50	2	4	46							1	3	
				50	3	2	48								2	
				50	4	3	47	1							1	1
				50	5	2	48				1					1
				50	6	3	47	2								1
				50	7	1	49	1								
Hogan	41.5	Rozol	3088	50	1	14	36	3			1	2		6		2
				50	2	10	40	1			3	2		2	2	
				50	3	4	46	1						3		
				50	4	3	47	1						2		
				50	5	2	48							2		
				50	6	2	48	1						1		
				50	7	0	50									

Table 5b. Amounts of visible bait post-treatment at various depths in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02), December, 2006, baiting.

Site Name	Acreage	Treatment	Burrows Baited	# Burrows Used to Assess Availability	# Days after Baiting	# Holes with Visible Bait	# Holes with no Visible Bait	## of Grains on Surface			## of Grains 0-6" below Surface			## of Grains >6" below Surface		
								<25	25-100	>100	<25	25-100	>100	<25	25-100	>100
Ryan South	24.4	Rozol	1787	50	1	34	16	1	1		4	6	10	4	3	5
				50	2	31	19	2			9	4	9	4	3	
				50	3	29	21	1	1		9	7	7	3	1	
				50	4	20	30				15	2		3		
				50	5	18	32				13	2		3		
				50	6	12	38				8			4		
				50	7	10	40				8			2		
Ryan Cemetery	14.5	Rozol	1503	50	1	32	18	1		1	2	6	14	3	2	3
				50	2*	26	24		1		5	3	11	1	2	2
				50	3	21	29				6	3	9		1	2
				50	4	16	34	1			6	3	2	2	2	
				50	5	15	35				7	2	2	2	2	
				50	6	13	37				7	4		1	1	
				50	7	12	38				6	4		1	1	

Table 5c. Amounts of visible bait post-treatment at various depths in 2006-2007 field efficacy trial for Rozol bait (Lee and Hyngstrom, 2007; MRID# 473336-02), March, 2007, baiting.

Site Name	Acreage	Treatment	Burrows Baited	# Burrows Used to Assess Availability	# Days after Baiting	# Holes with Visible Bait	# Holes with no Visible Bait	## of Grains on Surface			## of Grains 0-6" below Surface			## of Grains >6" below Surface		
								<25	25-100	>100	<25	25-100	>100	<25	25-100	>100
Sowers	4.8	Rozol	322	50	1	18	32				14	3		1		
				50	2	14	36	1			11	1		1		
				50	3	1	49					1				
				50	4	1	49				1					
				50	5	1	49				1					
				50	6	1	49				1					
				50	7	0	50									
Magnani	3.3	Rozol	101	50	1	31	19				14	9	2	2	4	
				50	2	27	23				11	9	2	2	3	
				50	3	14	36				4	7	2		1	
				50	4	10	40				3	6			1	
				50	5	7	43				2	5				
				50	6	5	45				3	2				
				50	7	3	47				2	1				
Wiese West	2.1	Rozol	174	50	1	35	15				17	7	8	3		
				50	2	31	19				13	7	8	3		
				50	3	31	19				13	7	9	2		
				50	4	10	40				4	3	3			
				50	5	5	45				3	2				
				50	6	2	48				1	1				
				50	7	2	48				1	1				
Wiese East	10.6	Rozol	435	50	1	25	25				17	3	4	1		
				50	2	28	22				12	3	2	1		
				47*	3	7	40				6		1			
				50	4	5	45				2	1	2			
				47**	5	1	46						1			
				50	6	3	47				1		1	1		
				50	7	2	48				1			1		

*On this plot on this day, one burrow was not scored for visible bait at all, while two others were scored as having visible bait present but without indicating the depth at which bait was visible.

**On this plot on this day, three burrows were scored as having visible bait; but the depth at which the bait was seen was not indicated.

Table 6. Comparison among treatment methods on depth of observable bait one day following treatment in Lee and Hyngstrom (2007) study.

Site Name	Month of Treatment	Treatment Method (s)	# Burrows Observed	Depth* at which Bait Was Observed: Surface	0-<6 Inches	6 Inches or More	No Bait Observed	% Holes with No Bait Observed	% Holes with No Bait <6 Inches Deep	% Holes w/Bait on Surface or <6 Inches Deep	% Holes with Bait on Surface
Sallee	October, 2006	Hand only	50	1	0	23	26	52.0%	98.0%	2.0%	2.0%
Hogan	October, 2006	Hand only	50	3	3	8	36	72.0%	88.0%	12.0%	6.0%
NE East Lashley	December, 2006	Hand only	50	1	2	27	20	40.0%	94.0%	6.0%	2.0%
Wiese West	March, 2007	Hand Only	50	0	24	11	15	30.0%	52.0%	48.0%	0.0%
Ryan South	December, 2006	Hand and Mechanical	50	2	20	12	16	32.0%	56.0%	44.0%	4.0%
NE West Faiman	December, 2006	Hand and Mechanical	50	2	12	13	23	46.0%	72.0%	28.0%	4.0%
Wiese East	March, 2007	Hand and Mechanical	50	0	20	5	25	50.0%	60.0%	40.0%	0.0%
Ryan Cemetery	December, 2006	Mechanical only	50	2	22	8	18	36.0%	52.0%	48.0%	4.0%
Sowers	March, 2007	Mechanical only	50	0	17	1	32	64.0%	66.0%	34.0%	0.0%
Magnani	March, 2007	Mechanical only	50	0	25	6	19	38.0%	50.0%	50.0%	0.0%

*As only one depth was reported for each burrow for each day, the depth indicated presumably was the shallowest depth at which bait was observed.