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SUBJ: EFED Evaluation of "A Probabilistic Assessment of the Risk of Brodifacoum to Nontarget Predators and Scavengers," by The Cadmus Group, September 10, 2004; Submitted by Syngenta Crop Protection, Inc.

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Attached is the Environmental Fate & Effects Division's evaluation of the Cadmus probabilistic assessment for brodifacoum, submitted by Syngenta Crop Protection for the brodifacoum registrants. It includes a more comprehensive review of the document performed by the Syracuse Environmental Research Associates and Syracuse Research Corporation for EFED and OPP. Please let us know if you have any questions you would like to discuss further with regard to these documents.

OPP Evaluation of Cadmus/Brodifacoum Registrants (C/BR) Probabilistic Risk
Assessment Model for Brodifacoum

Executive Summary

Brodifacoum is a second-generation anticoagulant rodenticide primarily used to control Norway and roof rats and house mice. In 1998, EPA issued a Reregistration Eligibility Decision (RED) for the rodenticide cluster, which included an assessment of human and ecological risks of brodifacoum and other rodenticides. However, the RED noted that EPA had received recent wildlife incident reports and that the Agency would continue to evaluate the risks of labeled uses of brodifacoum to nontarget birds and mammals. In 2001 (updated in 2004), EPA completed a comparative assessment of nine rodenticides used in the United States and concluded that brodifacoum labeled uses pose high potential primary and secondary risks to birds and nontarget mammals.

The 1998 RED and 2004 comparative assessment evaluated risks based on a lines of evidence and comparative-analysis model approach. In an attempt to estimate the probability and magnitude of potential ecological effects of brodifacoum, four rodenticide registrants (Bell Laboratories, Inc., LiphaTech, Reckitt Beneckiser, and Syngenta Crop Protection) contracted The Cadmus Group, Inc. to conduct a probabilistic ecological risk assessment. The Cadmus Group used a dietary dose model to develop distributions to estimate daily dose to nontarget predator species as a function of the body weight and food ingestion rate of animals, the concentration of residue in food, and the fraction of food in the diet containing brodifacoum. They also used an uptake-depuration model "to estimate the cumulative dose over time." Effects data used to develop the distributions were taken from published and unpublished sources. Finally, exposure and effects distributions were combined to estimate the probability of mortality to nontarget predator organisms. In their risk assessment, C/BR claimed low secondary risk of brodifacoum-induced mortality to coyote, red fox, and red-tailed hawk and inferred the same conclusion to other species of birds and mammals with similar sensitivity and diet. They also claimed that the secondary risk from brodifacoum was only slightly higher for the kit fox and great horned owl. Primary exposure of nontarget organisms was not addressed nor were risks to scavengers addressed explicitly. In characterizing the ecological risk of brodifacoum, C/BR acknowledged that risk estimates were limited by the lack of data and were subject to a number of uncertainties and assumptions.

After reviewing the C/BR probabilistic risk assessment of brodifacoum, EPA has concluded that the probabilistic risk assessment does not provide sufficient evidence to alter EPA's risk conclusions in the deterministic risk assessment. Similar to EPA's assessment, the C/BR assessment identifies information and data gaps that lead to major uncertainties in quantitatively assessing risks from secondary exposure to predators. The uncertainties identified in the C/BR report suggest that risks could range from a minimal likelihood of mortality to a high likelihood of significant mortality, depending on the extent to which predators consume rodenticide-exposed prey. Data that would help reduce the uncertainties in the risk assessments include information on local baiting practices, dietary composition and foraging behavior of birds and

mammals when bait and/or dead and dying animals supplement the natural food supply, toxicity data for predatory and scavenging species, concentrations of brodifacoum in target rodents and nontarget birds and mammals, and information on the retention, storage, and elimination of brodifacoum in nontarget birds and mammals. Because data are very limited for quantifying exposure under expected and typical use patterns, there is a large amount of uncertainty in the estimation of risk. As acknowledged in the C/BR assessment, risk depends strongly on local conditions and the foraging behavior and habitat use of predators and scavengers. Because of the spatial and temporal variability in all of these considerations, it becomes extremely difficult to quantify exposure and risk on a national scale.

1 Problem Formulation

General Conceptual Model

Although it was included in the initial draft of the problem formulation for the C/BR probabilistic assessment, primary exposure to nontarget animals that ingest bait was omitted in the final assessment. According to the C/BR assessment, primary exposure was excluded because (1) secondary exposure was of greater concern to regulators, (2) a different model would be needed to assess primary exposure, and (3) risk reduction for non-bait feeders is mostly a matter of bait station design. EPA has received data indicating that primary exposure to nontarget organisms does occur, and therefore, it is a concern for the Agency. Whether it is less or greater than secondary exposure is not as important as the fact that it does occur and should be addressed in the risk assessment. The need for another exposure model to address primary exposure is also not a reason to avoid assessing it. Thirdly, the Agency does not agree with the assertion that primary exposure is largely a function of bait station design, and thus, that it was appropriate to exclude primary exposure from this assessment.

Although the probabilistic assessment was designed to estimate probability and magnitude of potential ecological impacts, the scope of the C/BR assessment was limited. The initial problem formulation indicated that three scenarios would be considered: urban, suburban, and rural. Those scenarios were not addressed in the CB/R assessment, nor were any aspects of spatial or temporal variability.

The C/BR assessment of risks to predators examined exposure from ingestion only and, for the most part, ingestion of only Norway rats. Exposure from ingestion of roof rats, target and nontarget mice, birds, and invertebrates was not assessed. For example, residues might be higher or lower in mice than in Norway rats, and there are predators (e.g., kestrels, weasels) in use areas that might prey heavily on mice but take few, if any, rats.

Many predators and scavengers feed on those dead or dying birds that eat bait. For example, red-tailed hawks will prey on other birds (e.g., morning doves and starlings) which could be exposed to bait directly through the consumption of insects. Some of the studies in the source cited in the

C/BR report (e.g., U.S. EPA 1993) indicate that birds may comprise 10% or more of the hawk's diet. Canids (e.g., foxes and coyotes) will eat dead birds that have eaten bait, and one study found that birds comprised more than 48% of the diet (by frequency) of the coyote. This source of exposure needs to be considered in the risk assessment.

As acknowledged by C/BR, critical exposure data are lacking, which makes it difficult to quantify risk. For example, C/BR states that *PT* (the proportion of rodents in the diet that have been exposed to brodifacoum) is crucial to the model, but that "*We were unable to find any data regarding the proportion of rodents in the environment or in a predator's diet that may be exposed to rodenticides.*" As a result, the C/BR assessment relies on a series of assumptions, which are not well documented and which lead to a large amount of uncertainty in the risk conclusions. C/BR also states that *PD* (the proportion of rodents in the diet) and *PT* ". . . are largely determined by the behavior of the focal species and the characteristics of the habitat," but these factors are not considered in the model. Instead the assessment relied on LD50 values and average predator diets to derive risk curves for selected predators.

2 Effects Analysis

Quality of Data

Mammalian LD50 data were obtained for the dog, feral pig, guinea pig, mouse, rabbit, rat, sheep, and wallaby. Avian LD50 data were obtained for California quail, harrier hawk, chicken (two studies), Japanese quail, mallard, pheasant (two studies), and pukeko. The effects data were reviewed for quality only as it relates to the statistical aspects of the study. Methodological aspects of these studies were not examined. For example, the length of the study and supplemental vitamin K in the basal diet, both of which have been shown to affect toxicity of brodifacoum in laboratory tests, were not reported. Several of the avian tests were only available as sketchily described studies reported by Godfrey (1986)¹. Godfrey dosed different New Zealand bird species in 13 tests, but states that many of the results were inconclusive because of the small number of birds in several groups, problems in maintaining some species in cages, and the high interspecific and intraspecific variations known to occur with anticoagulant poisoning. The author goes on to call the results "*inconsistent*" and "*inconclusive*" and says his trials support the view that larger test groups are needed (e.g., only 3 test groups of 4 birds per group were used for the harrier hawk). "*Many of the birds had superficial wounds caused by contact with the cages and aviaries. Other birds did not adapt well to captivity and their feeding was irregular. Thus it is likely that several were under considerable stress which may have confounded the results obtained.*" C/BR explained that they teased data from the study and did not use results that were not appropriate. Such data were not used to calculate risk quotients in EPA's deterministic assessment and should not be relied upon in other than a qualitative manner in a higher-tier assessment unless the uncertainty is accounted for.

¹Godfrey, M.E.R. 1986. An evaluation of the acute-oral toxicity of brodifacoum to birds. Proc. Vertebr. Pest Conf. 12:78-81.

Sensitivity of Species

C/BR derived species sensitivity distributions (SSDs) separately for birds and mammals. They defined "low sensitivity" and "high sensitivity" species as those with LD50s at the 90th percentile and 10th percentile, respectively, of the distribution. (p. 19).

EPA is concerned not only with a large portion of the data used to generate the SSDs, but also with how the SSDs are used to generate risk probabilities. Whereas it is typical to use the 5th and 95th percentiles as the extremes of the SSD, C/BR instead used the 10th and 90th percentiles as indicators of "high" and "low" sensitivity, as described above. Because LD50 values for three of the nine bird species used by C/BR fall below the 10th percentile dose-response curve, the 10th percentile is not representative of a highly sensitive species. The risk assessment should note that data for other species not used also fall below the 10th percentile. For example, Godfrey (1986) reports LD50s <0.75 mg/kg for the Canada goose and black-backed gull. C/BR did not use or even cite those values because they were reported as "<". The values do provide information on the toxicity of brodifacoum, however, and should have been discussed in the risk characterization. Additionally, the available dietary (LC50) data and their potential impact on the species distributions should be discussed in the risk characterization, even if they were not used to generate the SSDs.

Representativeness of the Data

EPA questions how representative the SSDs are for nontarget animals that may be exposed to brodifacoum in North America. Much of the data were generated in New Zealand in the 1970s and consisted of LD50s for sheep, wallaby, European rabbit, Asian harrier hawk, and other species not occurring in North America. As discussed by Calow and Forbes (2003)², the fundamental assumption of representativeness of SSDs is violated if the data are from a small and biased set of species not present in the community or communities under threat. They also note that "*We rarely have enough information on the species sensitivities in particular communities to place much confidence in the precise risk probabilities generated by the SSD approach.*" Uncertainty, however, should be acknowledged and can be addressed, at least to some extent, using SSDs. Failing to do so is a weakness of model development and not of the SSD approach and probabilistic methods.

Canids as Surrogates

C/BR uses 3 surrogate species for mammalian predators and scavengers, and all are canids (coyote, kit fox, red fox). The only basis for using 3 canids as surrogates for all mammalian predators and scavengers seems to be that some dietary data were readily available (p. 15).

²Calow, P. and V.E. Forbes. 2003. Does ecotoxicology inform ecological risk assessment? Environ. Sci. Technol. April 1, 2003, pp. 147A-151A.

In their risk conclusions (p. 45), C/BR states that "By inference, the same conclusion applies to other species of birds and mammals with similar dietary composition and metabolism." Using other species besides canids (e.g., felid, mustelid) would have been more appropriate unless the assessment was intended solely to address risks to canids. Other generalist mammalian predators suitable for this assessment include the bobcat (*Lynx rufus*), long-tailed weasel (*Mustela frenata*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), and opossum (*Didelphis virginiana*). These species all have the ability to use a variety of habitat types but, nevertheless, they all differ considerably in their ecology and life history, and these differences are borne out in their differential responses to fragmented landscapes.³ EPA also notes that any of the surrogate canids might well ingest bait in addition to preying and scavenging on poisoned target and nontarget animals.

3 Exposure Analysis

Limitations of the Residue Data—Pulsed Baiting

C/BR states that "The availability of field data on residues in rodents greatly simplified the exposure analysis." (p. 17), "Our exposure analysis was based on residue data from carcasses, which we considered to overestimate the residue concentrations in live rodents captured by predators." (p. 15), and "Use of carcass residues overestimated exposure to predators, because brodifacoum concentrations in live rats were lower on average than concentrations in carcasses (Table 4)." (pp. 26-27).

These statements imply that adequate residue data are available to characterize residues in dead and dying target species exposed to brodifacoum, and secondly, to determine whether or not they overestimate residues found in live rodents. The residue data used in the assessment come entirely from three field studies conducted on farms in the U.K. in the early 1980s⁴ and almost all are for the Norway rat. In addition, most of the residue data were obtained from rats which were exposed to 'pulsed-baiting' applications, which differs markedly from the 'saturation' or

³Gehring, T.M. and R.K. Swihart. 2003. Body size, niche breadth, and ecologically scaled responses to habitat fragmentation: mammalian predators in an agricultural landscape. *Biol. Conservation* 109:283-295

⁴Edwards, P.J. and H. Swaine. 1983. Hazard to non-target animals from the use of 'Klerat' bait on farms in the UK for control of the common rat *Rattus norvegicus*. Report no. RJ0305B. ICI Plant Protection Division, Berkshire, UK. 31 pp.

Edwards, P.J., H. Swaine, and S.H. Kennedy. 1984. Hazard to non-target animals from 'pulsed' baiting with 'Klerat' pelleted bait around farms buildings. Report no. RJ0369B. ICI Plant Protection Division, Berkshire, UK. 53 pp.

Edwards, P.J., H. Swaine, J.M. Coulson and S.H. Kennedy. 1984. Hazard to non-target animals from 'pulsed' baiting with wax block baits around farm buildings. Report no. RJ0375B. ICI Plant Protection Division, Berkshire, UK. 57 pp.

'sustained' baiting done in the U.S. and on several of the farms in the U.K. Kaukeinen (1993)⁵ states that "*Pulsed baiting is a technique of placing limited amounts of rodenticide at timed intervals, rather than maintaining a surplus. This may extend the time to control and increase labor (which may be unacceptable for some use patterns), but the method can reduce the quantity of toxicant placed in the environment at any given time.*"

In the pulsed baiting in the U.K. studies, 1-2 oz. (20-50 g) of bait was placed at 15- to 30-ft (5-10 m) intervals, and bait was replenished only at 7-day intervals. In contrast, product labels in the U. S. direct applicators to apply 4-16 oz of. bait at 15- to 30-foot intervals and to maintain an uninterrupted supply of fresh bait until signs of rat activity cease. A rat can ingest a lethal dose in a single feeding but, on average, it does not die until 6 days later and will continue to eat bait. By applying smaller amounts of bait and replenishing only at 7-day intervals, the technique of pulsed baiting results in individual rats consuming lesser amounts of bait before dying, thereby reducing the total amount of bait needed for control (Dubock 1982)⁶. In contrast, saturation baiting allows individual rats to feed on bait continually until they die, thereby increasing the amount of brodifacoum ingested and increasing residue levels in the body. Regarding the residue data collected in these U.K. studies, Kaukeinen (1993) states that "*Pulsed baiting produced more carcasses containing lower residues than typical, sustained baiting.*"

C/BR claims (p. 26) that the residue data of Edwards, et al. were analyzed for differences among the baiting practices (pulsed vs saturation) and that no significant differences were found. Therefore, the rat data were pooled along with residue data for 4 house mice, 4 wood mice, and 1 vole subjected to pulsed-baiting. EPA has examined the residue data for the rats analyzed in the three Edwards studies. Of 138 rats analyzed, 111 were from farms which were pulse-baited at 7-day intervals and 27 from farms which were saturation-baited. However, data from one farm needs to be excluded, because other anticoagulant rodenticides (coumatetralyl, warfarin) were used concurrently with brodifacoum but were not accounted for in the residue analyses. Excluding those, residue data are available for 22 rats subjected to saturation baiting. The mean residue level in the 111 pulsed-baited rats was 1.6 ppm, versus 3.4 ppm in the 22 saturation-baited rats. These values are significantly different ($t = -2.52, p < 0.01$) and clearly should not be pooled. Even if the data were not significantly different, there is no justification for using residue data from rats exposed to pulsed-baiting applications. Pooling the pulse-baited rats with the saturation-baited rats reduces the mean residue level from 3.4 ppm to 1.9 ppm. This, in turn, leads to predictions of lower exposure and therefore lower risks to predators and scavengers.

⁵Kaukeinen, D.E. 1993. Nontarget organism evaluations for rodenticides. Pages 352-363 in K.D. Racke and A.R. Leslie (eds), *Pesticides in Urban Environments: Fate and Significance*. Developed from a symposium sponsored by the Division of Agrochemicals at the 203rd National meeting of the American Chemical Society, San Francisco, California, April 5-10, 1992

⁶Dubock, A. 1982. Pulsed baiting -- a new technique for high potency, slow acting rodenticides. *Proc. Vertebr. Pest Conf.* 10:123-136.

Omitting the pulsed-baiting studies from the assessment would leave field data from only 22 rats exposed with any similarity to baiting practices in the U.S. Consistent with the comments EPA has made repeatedly in communications with the registrants, this is a very minimal dataset contributing to uncertainty in the assessment.

Residue Reporting Data

The data from 13 rats captured alive and analyzed for brodifacoum residue in Edwards et al. (1984) was incorrectly reported as being "0.05 ppm (mg/kg)," which was the Limit of Detection for brodifacoum. By omitting the "<" and reporting the residue level as "0.05 mg/kg" for each of those rats, the assessment implies that a low level of residue was detected. This is probably not the case. In fact, if no residue was detected in 5 rats, it may indicate that the baiting program was not very efficacious or that the rats had not yet eaten the bait.

Exposure Model

The C/BR dietary dose model is specified as follows:

$$DD = FIR \times PD \times PT \times C$$

where

- DD = Daily dose to the nontarget predator
- FIR = food ingestion rate of the predator
- PD = % rodents in diet of the predator
- PT = % rodents exposed to rodenticide
- C = concentration of brodifacoum in exposed rodents

$$FIR = FMR / (GE \times AE \times M \times Wt)$$

where

- FMR = field metabolic rate (function of log(a), b, and Wt) of the predator
- GE = gross energy content of food (rodent)
- AE = assimilation efficiency
- M = moisture content of food
- Wt = body weight

Limiting Assumptions for PD and PT

Two important components of the exposure model are the proportion of rodents in a predator's diet (PD), and the proportion of exposed rodents in the predator's diet, (PT). Regarding PD and PT, C/BR states (p. 17) that "*Both variables are largely determined by the behavior of the focal species and the characteristics of the habitat.*" and PT is "*... assumed equal to the fraction of time spent in the treated area.*"

Behavior and habitat are not addressed in the model. Instead, C/BR uses field data on dietary composition as reported in the literature. This may be a problem since most of the dietary studies in the literature were conducted in wild areas (e.g., boreal forest, prairie, fields, woodlots, etc.) devoid of commensal rats and mice, and thus commensal rodents comprised little if any of the diet. Secondly, when a rodenticide bait is applied, the food supply is altered in two ways. The bait provides a supplemental food source for target and nontarget primary consumers, which in turn provides a source of dead and dying food for predators and scavengers. Opportunistic species, including the five surrogate species used in the model, may drastically alter their foraging behavior and food habits to exploit an abundant, even if ephemeral, food source. C/BR does acknowledge this on p. 24 where they state that "*many predators, including the five focal species we modeled, are opportunistic, and their diet reflects available food supply.*" Although the assessment acknowledges that exposed rodents are more susceptible to predation than unexposed rodents, the model did not account for this in the predator's diet composition.

Incorporating feeding behavior and other aspects affecting it into the model may be more difficult than ignoring it, but it can be done. In their submission of February 23, 2004, C/BR states that FIR and PD could be adjusted to vary seasonally to reflect changing feeding habits of a predator species; the distribution of C [Concentration in exposed rodents] could be adjusted to represent increased predation on moribund rodents, changes in dosing regimens, and changes in the concentration in rodents as a function of time; PT could fluctuate cyclically to represent different baiting practices; and time dependencies could be incorporated."

C/BR states in the Results (p. 38) that ". . . risk increased sharply with increasing PT." and "*In situations where a high percentage of rodents are exposed to brodifacoum, risk of secondary poisoning may become high even for predators with a relatively low proportion of rodents in their diet.*" Moreover, in their submission of February 23, 2004⁷, C/BR states that ". . . we will run the model using different assumptions based on indirect estimates and expert opinion (including PT=1 as the worst case)." For some reason, higher-exposure scenarios were not presented in the assessment. In the Summary Table on p. 3, however, C/BR presents estimated mortality rates for the five surrogate species, assuming median sensitivity and only 1% exposed rodents in the diet. Results of exposure scenarios using PT values above 2.5% (e.g., 15%) were not included in the risk assessment even though the C/BR acknowledged that they "*compared results of the daily dose model using PT values ranging from 1% to 15%.*"

In contrast to its stated results at the end of the document, C/BR writes at the beginning of the report that "*The risk of brodifacoum-induced mortality is low in the coyote, red fox, and red-tailed hawk, and by inference the same conclusion applies to other species of birds and mammals with similar dietary composition and metabolism. Risk is slightly greater for species with a higher percentage of rodents in their diet, such as the kit fox and great horned owl.*" "Slightly"

⁷Methods and Procedures For Assessing the Risk of Rodenticide Baits Containing Brodifacoum to Non-Target Animals, W. Warren-Hicks and J. Giddings, The Cadmus Group, February 23, 2004, 5 pp.

higher for the kit fox means that there is a 10% probability that mortality exceeds 5% under those assumptions and exceeds 7% for the great horned owl. Apparently, mortality could be as high as 100% for all species.

Limiting the estimate of the range of such a sensitive parameter as PT to an unsupported assumption of 1% is not appropriate. As suggested below, assumptions concerning values for PT could be based on the assumption that brodifacoum is being used in an effective baiting program and that both target and nontarget rodents may be exposed.

On p. 28 C/BR states that *"The proportion of Norway rats and house mice that are exposed to a brodifacoum baiting program is unknown, but is likely to be much smaller than one in ten."*

The value of one in ten is unsupported and seems to postulate an ineffective baiting program – i.e., one where only a small proportion of the target organisms will be exposed. Assuming that brodifacoum is used effectively – i.e., a large proportion of the target rodents are killed by exposure to brodifacoum, it follows that a large proportion of the target rodent population is exposed. This assumption is reinforced by the Edwards studies (Edwards and Swaine 1983; Edwards et al. 1983, 1984a,b) which also suggest that substantial numbers of nontarget species are likely to be exposed in an effective baiting program, particularly if saturation baiting methods are used.

As a preliminary illustration, Edwards et al. (1984b, cited in footnote 4) provide data on a relatively successful baiting program – i.e., p. 20, Table 8, Farm A. In this example, control of rats is estimated at 88%. In other words, it seems safe to assume that at least 88% of the targeted rats were exposed – i.e., $PT = 0.88$, rather than 1%, for effective control of a rodent infestation. As discussed by Edwards et al. (1984b), the control rate of 88% is probably an underestimate because of reinfestation. A more careful review of data associated with successful baiting programs would probably result in better estimates of PT and possible error ranges associated with PT. EPA asserts that plausible estimates of PT (which should encompass both target and nontarget rodents) are substantially higher than the 0.01 to 0.15 values used by C/BR.

Section 3.2.2.5 further describes PT as the portion of consumed rodents that are exposed to brodifacoum. In the daily dose model, PT is implemented as a binomial distribution, where a value of 0 indicates that none of the rodents consumed contain brodifacoum and a value 1 indicates that all of the rodents consumed contain brodifacoum. All or nothing is an extreme scenario.

The authors state (p. 25), *"Because a single rat would represent a substantial fraction of the daily food intake of an individual predator, intermediate values of [daily] DPT would be unlikely; that is, the day's rodent intake would be either entirely exposed or entirely unexposed."* This assumption should be explored more fully, especially for target and nontarget mice which are much smaller than rats. The distribution of FIR should be presented together with the distribution of body weights for rodents.

The consequence of the "all or nothing" modeling approach for determining daily doses (given the small PT value used by the authors), is that a high percentage of the exposure days yield a dose of 0, and a small percentage yield an extremely high dose. The likelihood of exposure, or equivalently, the average percentage of days in which exposure is non-zero is determined by PT, which the authors have claimed to be very small.

The authors of the report appended to this review suggest the alternative approach of applying PT to individual prey meals rather than to total daily events, such that the conceptual model simulates "prey consumption events" rather than "total daily events." As they note, this gives a greater chance that at least one meal is contaminated, so there will be fewer days with doses of 0. Also, "unlike the 'all or nothing' case that is modeled right now, it is less likely that ALL of the prey items [consumed per day]...[would or would not be] contaminated on any given day."

As demonstrated on pages 35-39 of the Appendix, for very low values of PT (e.g., $PT < 20\%$) and very high values of PT (e.g., $PT > 90\%$), the two approaches yield essentially the same result, as one would expect. This is because the frequency of daily consumption of contaminated prey is represented by extreme cases. However, for intermediate estimates of PT (e.g., $30\% < PT < 70\%$) the difference can be significant. While the mean daily dose (DD) is approximately the same, less than 25% of the daily estimates are 0 for the individual prey method. (See Figures 1 and 2 and Table 1.)

Key to the impact of this alternative is the bioaccumulation potential of brodifacoum. The difference in modeling approaches (i.e., All or Nothing vs. Individual Prey methods) may be important if the half-life for elimination kinetics is moderate to long (> 10 days), as is the case for brodifacoum. The greater the frequency of consecutive days of non-zero exposure, the more brodifacoum can be expected to accumulate in the body of the predator or scavenger.

Distribution of 100 Means v. Distribution of 10,000 Exposure Events

C/BR calculated a summary statistic from the time series of body burdens. They suggested that the 90-day maximum is an appropriate metric. For each individual, 100 sets of time series were generated, with each time series yielding an estimate of the 90-day maximum. Therefore, for 100 individuals, there were 100 sets of time series of 90-day maxima.

At this point, there are two possible approaches to generating the distribution of doses that represents the combined sources of variability in the model:

1. **Summary Statistic Approach.** For each individual, calculate a summary statistic from the 100 sets of 90-day maximum. For example, calculate the mean 90-day maximum, or calculate an upper percentile (e.g., 95th %ile), or even the maximum of the maximum. The choice clearly has important implications for the overall distribution of doses and risks to the population.

2. **All 90-day Maxima Approach.** Use all of the 100 values of 90-day maxima for all 100 individuals, yielding a data set of 100 x 100 or 10,000 values to define the exposure (dose) distribution.

Section 3.4.4 of the report describes the approach, stating that *"The mean of the 100 simulation outcomes was calculated, and the 100 means (from 100 individuals) were plotted as reverse cumulative frequency distributions."* The risk characterization (Section 5.1), however, states, *"For each cumulative dose model run, the full set of 10,000 dose estimates (100 estimates for each of 100 individuals) was combined into a single exposure distribution reflecting both within-individual and among-individual variation."*

While the statements are conflicting and the input files were not included in materials provided, it appears from code and from certain descriptive statistics that the C/BR assessment elected Option 1, with the summary statistic being the **arithmetic mean** of the 90-day maxima. This yielded a data set of 100 values of mean 90-day maxima.

EPA maintains that the difference between the distribution of 100 means of 90-day maxima, and the distribution of 10,000 values of 90-day maxima is a large one. The former could greatly underestimate the upper tail compared with the latter. Figures 7 and 8 of the Appendix provide an example for coyote. For this example, setting $PT = 0.025$, $half\ life = 5$ days, and $SD\ of\ DPD = 5\%$, the effect of decreasing variability by representing individuals by the mean 90-day max is to decrease the dose at the 5% exceedance level by 40%.

90-day Time Period for Exposure

The C/BR assessment claims that *"In each of the 100 inner loop iterations, a sequence of 90 independent daily doses was simulated ..."*

The choice of a 90-day period appears to be arbitrary. C/BR elected to select the first 90-day period for each time series of daily doses. All individuals began the time series with a body burden of 0 mg/kg. The concept of first order kinetics implies that it may take some time for the chemical to achieve steady state. This time to steady state is influenced by both the choice of depuration half-life ($t_{1/2}$) as well as the frequency of daily doses. It should be noted that for some scenarios, the frequency will be very low because PT is set to a low percentage, and because, as mentioned above, it is applied as an "all or nothing" approach.

The difference between the 90-day maximum during the first 90 days and the maximum during the last 90 days of a 365 day period is demonstrated in Figures 9-11 of the Appendix. These authors make the following observations:

- When the dosing regimen is frequent, the assumption about $t_{1/2}$ becomes critical; and
- The maximum 90-day body burden is unlikely to be captured by the first 90-days of the

time series, especially for longer half-life scenarios.

Kinetic model

C/BR's kinetic model is limited to one compartment and does not consider available metabolism studies (See detailed comments in the Appendix.) C/BR postulates a single compartment kinetic model for brodifacoum, claiming that "*Depuration kinetics of brodifacoum are complex, as described in Sections 2.2.3 and 3.3.2.2. Studies in rats suggest that depuration follows a biphasic curve (Kaukeinen et al. 2000). The initial phase of the curve is steep, with a half-life on the order of a few days, reflecting a pool of rapidly eliminated brodifacoum in the body. The second phase of the depuration curve is much slower, with a half-life on the order of many months, reflecting a much smaller pool of tightly bound brodifacoum in the liver. However, the toxicokinetics of brodifacoum, particularly the rates of exchange between blood, liver, and other tissues, have not been quantified even in the rat. Many mechanistic and quantitative assumptions would be needed to simulate the biphasic depuration process, and the results would reflect large uncertainties in these assumptions. ...*"

The latter two statements give the impression that the kinetics of brodifacoum are poorly understood and cannot be used for the type of analysis being considered by C/BR. Information on the pharmacokinetics of brodifacoum is available in 16 studies on seven species. These studies are discussed in the Rodenticide Cluster RED⁸ and in EPA's comparative risk assessment for the rodenticides.

C/BR's discussion of a "*pool of rapidly eliminated brodifacoum*" (p. 21) appears to be a misinterpretation of the biphasic pattern in the concentration of brodifacoum in the body. The first and more rapid phase is associated with redistribution from blood to tissue, not excretion.

The C/BR report chose a range of half-lives from 2 days to 200 days with the implication that the "real" half-lives are somewhere in this region. In terms of the body burden of the prey species, EPA believes that the shorter half-lives of 2 days to several days reflect the blood data and redistribution from blood to tissue, but do not reflect body burden. The upper range of 200 days is presumably based on liver half-lives. As reviewed in EPA's rodenticide risk assessment, reported liver half-lives are in the range of about 74 to 350 days.

The assessment then went on to discuss the use of the first order model and a sensitivity analysis using the range of values (2 to 200 days). As discussed in detail in the Appendix, the sensitivity analysis may lead to the impression that the kinetics have minimal impact on the assessment of risk. Basing most of the analyses on a 50-day half-life in a simple one compartment model, however, will probably underestimate risk.

⁸Reregistration Eligibility Decision (RED): Rodenticide Cluster. July, 1998. EPA738-R-98-007. 307

As illustrated in the Appendix, the kinetics of brodifacoum can be described by a simple flow-limited physiologically based pharmacokinetic (PBPK) model that is optimized with concentration data in liver, kidney, and carcass. Such a model accounted for the bulk of the brodifacoum residue data in the rat.

Field and Incident Data

Relevant information that could be used as data inputs for the exposure model is available. For example, there is an ongoing field project involving the San Joaquin kit fox⁹, one of C/BR's surrogate species, that could have provided more relevant and useful information than the few dietary studies C/BR gleaned from the open literature. Also in California, researchers associated with the Santa Monica Mountains National Recreation Area, Department of Natural Resources Conservation, and the University of California at Los Angeles have studied the ecology and behavior of coyotes (also a surrogate species) and bobcats relative to development in a fragmented habitat in southern California for several years¹⁰. They captured and radiocollared numerous coyotes, bobcats, and mountain lions and determined home ranges in relation to human development. Many of these individuals were exposed to anticoagulant rodenticides, including brodifacoum.

In addition, the C/BR risk characterization could include an explanation of the incident data that are available on brodifacoum. This data was summarized in EPA's rodenticide risk assessment as well as incident data reported in publications cited in the C/BR report (e.g., Edwards and

⁹Dr. B. Cypher, Research Ecologist for The Endangered Species Recovery Program (ESRP), coordinates several of ESRP's research projects on San Joaquin kit foxes. The ESRP is a cooperative research program on biodiversity conservation in central California, administered by California State University, Stanislaus Foundation. Its mission is to facilitate endangered species recovery and resolve conservation conflicts through scientifically based recovery planning and implementation. The program was established in 1992 at the request and with the support of the U.S. Fish and Wildlife Service and the U.S. Bureau of Reclamation. Over the past decade, ESRP has grown into a cooperative research program working with local, State, and Federal agencies, non-governmental organizations, corporations, and private land owners.

¹⁰e.g., see S.P.D. Riley et al. 2002. Effects of Urbanization and Habitat Fragmentation on Bobcats and Coyotes in Southern California. *Conservation Biol.* 17:566-576 and in prep

Swaine 1983; Edwards et al. 1983a,b) and in the open literature^{11,12,13,14}

4 Risk Characterization

Implementation

While employing a Bayesian hierarchical framework is an interesting and potentially useful innovation, the use of such a model is not a substitute for limited, incomplete, and inconsistent data. Further, the model is implemented using certain assumptions that have the potential to severely limit the range of possible predicted outcomes.

On page 35 the authors state that “*hierarchical models reduce the effect of incomplete data sets, small numbers of tests, inconsistent information on effects among species, and other issues that lend uncertainty to the effects models.*” While EPA agrees that this can be a helpful approach in such situations, the extent to which the limitations of incomplete data can be addressed by such a modeling approach is not clear in general. In the specific case of the C/BR assessment where the range of species selected is limited, where only certain studies within species have been selected, where available metabolism data have not been used, and where assumptions on behavioral parameters have not been explored to the full range of their implications for exposure, the benefit of such an approach cannot be expected to overcome the limitations of the data.

Furthermore, the selection of the 10th percentile to constitute a “sensitive” species, especially among those particular species assembled, does not necessarily constitute a conservative approach, but rather, appears to limit severely the resulting overall effects distribution when combined with the median and 90th percentile species estimates. As pointed out in the SERA/SRC report appended to this document, the “bounds shown in Figures 15 and 16 do not appear to represent the variability that exists, even within these very limited data sets, and should not be used to define what the dose-response relationship would be for a hypothetical sensitive nontarget species. The bounds do not include even 80% of the dose-response curves used. For birds, out of 9 curves, only 3 are completely contained within the 80% confidence bounds. For mammals,...Of the 8 curves shown, only 1 is completely contained within the 80%

¹¹Stone, W.B., J.C. Okonlewski, and J.R. Stedelin. 2003. Anticoagulant rodenticides and raptors: recent findings from New York, 1998-2001. Bull. Environ. Contam. Toxicol. 70:34-40

¹²Stone, W.B., J.C. Okonlewski, and J.R. Stedelin. 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. J. Wildl. Diseases 35:187-193

¹³Hosea, R.C. 2000. Exposure of non-target wildlife to anticoagulant rodenticides in California. Proc. Vertebr. Pest Conf. 19:236-244

¹⁴S.P. D. Riley et al. 2002. Effects of Urbanization and Habitat Fragmentation on Bobcats and Coyotes in Southern California. Conservation Biol. 17:566-576

bounds." (p.10) An examination of these figures therefore indicates that even with the limited data chosen, this implementation of the hierarchical approach appears to restrict, rather than encompass or expand, the range of outcomes expected.

Finally, factors such as distribution assumptions for both the model parameters of the overall distribution and for the individual species dose-response curves should have been discussed in the C/BR assessment. Although the assumptions for the 'super' distribution the parameter values may be typical, their impact should be explored, especially given the results in the figures mentioned above. Additionally, while the impact of using a logistic model rather than the probit in analyzing the dose-response data might not be large with respect to the LD50, it may underestimate mortality at the lower end of the curves.

Results and Conclusions

The C/BR exposure characterization is based on a series of unsupported assumptions that contribute to a highly uncertain risk estimate. C/BR provides exceedence probabilities (Table 14, p. 70) assuming high (10th percentile), median (50th percentile), and low (90th percentile) sensitivity and either 1% or 2.5% exposed rodents in the diet. For the kit fox, assuming high sensitivity and 2.5% exposed rodents in the diet, there is a 10% probability that mortality exceeds 67% and a 50% probability that mortality exceeds 25%. C/BR categorizes that risk as "slightly greater" than the "low" risk identified for other surrogate species. Even assuming only 1% exposed rodents in the diet and high sensitivity, there is a 10% probability that mortality exceeds 23%. Exceedences for the great horned owl are nearly as high. Exceedences are even higher as the percentage of exposed rodents increases in the diet.

On p. 31 C/BR states that "*Circumstances in which PT is greater will result in proportionally greater daily doses.*" While in the model this does not necessarily correlate directly with proportional estimates of mortality, there is some indication from Table 14 that mortality may increase at a higher rate, depending on species and assumption of sensitivity. Nevertheless, it is clear that a 6-fold increase in PT from 0.025 to only 0.15 would result in a substantially higher probability that mortality will be close to 70%. Alternatively, the same change in PT would result in a 50% probability of far greater than 25% mortality for kit fox under the circumstances cited above.

Finally, field data would appear to support these higher assumptions for exposure. Low-to-no predictions of risk in C/BR's assessment are not substantiated in the field, where field studies and incident reports clearly indicate that risks to nontarget species are not only plausible but are well-documented.

Uncertainty

Although the C/BR assessment acknowledges the limitations and uncertainties associated with the assumptions inherent in their assessment of brodifacoum risk to scavengers and predators, a quantitative uncertainty analysis or bounding analysis to improve the risk characterization is not

provided. Had the variables that C/BR summarized been addressed (or followed through) in the problem formulation stage of the effort, a more useful product for risk managers might have been developed. These include limitations of scope from restricting the assessment to secondary exposure with one target species, failing to consider spatial/temporal variations such as habitat and season, and a more detailed explanation of PT and PD.

Finally, as previously emphasized in this evaluation, a major issue in the C/BR assessment is the lack of quantifiable exposure data. Data which can provide a more solid foundation for estimating exposure would be extremely useful to reduce uncertainty in risk estimates. Where data are limited, however, methods are available in the implementation of the model for addressing uncertainty. A major deficiency with the current model which severely limits its utility is its failure to address the important variables mentioned above and to account adequately for uncertainty in the parameters it does address.



**An Exploratory Physiologically Based
Pharmacokinetic Model for Brodifacoum**
Attachment to: *Peer Review of Brodifacoum (PP581) Assessment*
WA 2-10, SERA TR-46-2-10-1e

Submitted to:

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Table of Contents

1. Introduction 1

2. Materials and Methods 1

 2.1. Pharmacokinetic Study in Rats 1

 2.2. Model Structure 2

 2.3. Parameter Estimates 3

3. Results 4

4. Discussion 5

5. References 7

Appendix: Model Source Code in ACSL

LIST OF FIGURES

Figure 1: Overview of PBPK Model for Brodifacoum Figure-1

Figure 2: Comparison of observed and modeled concentration in the liver, kidney,
and carcass for the mid dose group Figure-2

Figure 3: Comparison of observed and modeled concentration in the liver Figure-3

Figure 4: Comparison of observed and modeled concentration in the kidney Figure-4

LIST OF TABLES

Tables 1: Physiological Parameters Used in PBPK Models Tables-1

Table 2: Selected Properties of Brodifacoum Tables-2

Table 3: Estimated first-order rate constants Tables-2

NOTE: Tables and Figures are placed after the appendix

1. INTRODUCTION

This brief report presents an exploratory and preliminary physiologically based pharmacokinetic (PBPK) model for brodifacoum. This exercise is motivated by the review of a probabilistic risk assessment on brodifacoum (Cadmus 2004) that was submitted to the Environmental Fate and Effects Division (EFED). The Cadmus (2004) report states that *...the toxicokinetics of brodifacoum, particularly the rates of exchange between blood, liver, and other tissues, have not been quantified even in the rat* and bases their probabilistic risk assessment on a classical single compartment kinetic model. The nature of the available pharmacokinetic studies, however, were not detailed.

In order to obtain a better understanding of the limitations in the pharmacokinetic studies on brodifacoum, two reviews on brodifacoum (Erickson and Urban 2004; WHO 1995) were consulted and a preliminary literature search was conducted on TOXLINE (<http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE>). Information was identified on the pharmacokinetics of brodifacoum in 16 studies on seven species: rats (Bachmann and Sullivan 1983; Batten and Bratt 1990; Belleville 1991; Bratt and Hudson 1979; Hawkins 1991; Parmar et al. 1987), possum (Eason et al. 1996), rabbits (Breckenridge et al. 1985), sheep (Lass et al. 1985), dogs (Robben et al. 1998; Woody et al. 1992), horses (Boermans et al. 1991; McConnico et al. 1997) and humans (Bruno et al. 2000; Hollinger and Pastoor 1993; Weitzel et al. 1990).

Based on the summary of most of these studies in Erickson and Urban (2004), full copies of two studies were obtained: Batten and Bratt (1990) and Lass et al. (1985). The study in sheep by Lass et al. (1985) did not contain many experimental details and was conducted using only two dose levels. While this study might be useful in assessing the species scalability of a PBPK model for brodifacoum, it did not appear to be suitable for the development of a PBPK model, and this study was not pursued further. The study by Batten and Bratt (1990) is a relatively standard pharmacokinetic study submitted to support the registration of brodifacoum. As detailed below, the study provided sufficient information for the development of a preliminary PBPK model for brodifacoum.

2. MATERIALS AND METHODS

2.1. Pharmacokinetic Study in Rats

The study by Batten and Bratt (1990) involved groups of male rats (about 7 weeks old and weighing between about 180 and 220 g). The rats were administered C¹⁴-brodifacoum by gavage at doses of 0.02 mg/kg, 0.15 mg/kg, or 0.35 mg/kg. Animals were observed and assays of brodifacoum were made for up to 104 days.

Concentrations of brodifacoum were determined (by serial sacrifice) in the liver, kidney, salivary glands, and pancreas in all three dose groups. Concentrations of brodifacoum were determined in the blood and fat in the high-dose group and the carcass in the mid-dose group. Attempts were made to determine the concentration of brodifacoum in the blood of animals in the low-dose and

mid-dose groups but the concentrations were below the limit of detection. Even at the high-dose group, concentrations in the blood could not be detected after Day 8. Measurements of the proportion of the dose excreted in urine and feces were made by 24-hour collections before sacrifice at various times in the low-dose and mid-dose groups. Except for values in the mid-dose groups at Day 1, amounts in the urine were below the limit of detection. In the low-dose and mid-dose groups, the majority of the brodifacoum was excreted in the feces. In both groups, there was a very high rate of fecal excretion at Day 1. This is not discussed by Batten and Bratt (1990) but may simply be an artifact of gavage administration.

As discussed by Batten and Bratt (1990), the two lower doses were considered non-toxic. No adverse effects were noted and no effect was seen on clotting times or body weight gain. In the high-dose group, clotting time was increased from about 24 hours to 96 hours after dosing. Also in the high-dose group, body weight gain was depressed, some of the rats died, and other rats evidenced signs of internal hemorrhaging. All of these effects in the high-dose group were attributed to brodifacoum.

Details of the observations from Batten and Bratt (1990) that were used or considered for quantitative analyses are included in the worksheet titled **Batten et al 1987 Data** in the EXCEL workbook, **Batten and Brat 1990 Data.xls**. A copy of this workbook is included with the current report. Batten and Bratt (1990) report data in nanomoles/gram of tissue (equivalent to $\mu\text{moles/kg}$ tissue). In the development of the PBPK model, all concentrations were transformed to units of mg/kg or mg/L. Details of these conversions are given in the workbook.

2.2. Model Structure

An overview of the PBPK model for brodifacoum is given in Figure 1 and the source code for the model is given in Appendix 1.

Brodifacoum was modeled with a standard blood flow-limited system:

$$\frac{dA_o}{dt} = V_o \frac{dC_o}{dt} = Q_o C_B - Q_o \frac{C_B}{P_o} \quad (\text{Eq. 1})$$

In Equation 1, the change in the amount of a substance in an organ per unit time (dA_o/dt) can be defined equivalently as product of the volume of the organ (V_o) and the change in the concentration of a substance in an organ per unit time (dC_o/dt). The amount entering the organ at time t is simply the rate of blood flow to the organ (Q_o in L/day) and the concentration in the afferent or arterial blood (C_B in mg/L). The concentration in the efferent or venous blood is calculated as the concentration in the arterial blood divided by the organ-blood partition coefficient (P_o). This type of flow-limited transport assumes that movement across the cell membrane is very rapid relative to the rate of blood flow. This assumption is generally

applicable to lipid soluble, unionized, low molecular weight compounds - i.e., compounds that are likely to be rapidly absorbed across cell membranes (Gibaldi and Perrier 1982).

Excretion from the kidney and liver is modeled as a first-order process. Excretion from the liver is directed to the gut lumen and excretion from the kidney is directed to the urine. The gut tissue is modeled as a standard flow-limited compartment with absorption from gut lumen and stomach lumen. Absorption from the gut lumen and stomach lumen is treated as a first-order process. Movement from the stomach lumen to the gut lumen and from the gut lumen to the feces is also modeled as a first order process. While enterohepatic recirculation is not discussed in the study by Batten and Bratt (1990), enterohepatic recirculation seems plausible for a highly lipophilic compound like brodifacoum and was incorporated as resorption from the gut lumen to gut tissue with subsequent transfer back to the liver.

Preliminary attempts to optimize liver tissue clearly suggested that the standard flow-limited model did not well describe the biphasic pattern over time in the concentration of brodifacoum in the liver. Thus, as a first approximation, a standard deep compartment was added to the liver in which transport to and from the deep compartment was modeled as a first-order process - i.e., the parameters $k_{1,2}$ and $k_{2,1}$.

2.3. Parameter Estimates

The physiological parameters used in the PBPK models are summarized in Table 1. Most parameters, including the organ volume and flow, are taken from ILSI (1994); the other values are taken from Davies and Morris (1993). The fractional weight and fractional flow for the carcass was set to 1 minus the sum of the fractional weights of the other organs. In other words, the fractional organ weights (including the blood) always sum to unity. Similarly, the proportion of cardiac output is always set so that the sum of the fractional flows equals unity.

As noted above, transport from the stomach lumen to the lumen of the gastrointestinal tract is explicitly modeled. To avoid having to optimize this parameter, the value was set to the reciprocal of the transit time for the stomach, as reported by Davies and Morris (1993). Similarly, rather than optimizing the first-order coefficient for fecal excretion, the value was set to the reciprocal of the GI transit time in rats, also from Davies and Morris (1993). Neither of these estimates required adjustment.

The pharmacokinetic models were developed, solved, and optimized using ModelMaker 4 software (Cherwell Scientific Limited, 2000) with Gear's method for numerical integration. Although Batten and Bratt (1990) provide individual animal data, mean values were used in this preliminary analysis. Initial model parameters were set by eye fit. Final estimates prior to optimization were set by simulated annealing. Model optimization was performed using a standard Levenberg-Marquardt algorithm with an error weighting of 0.2. This weighting factor was based on a cursory examination of the variability of the individual animal data. In a more detailed analysis, the individual animal data would be used. The model was also ported to ACSL (Aegis Technologies 2004) and the source code for the model in Appendix 1 is given in ACSL.

The ModelMaker source code is in the file **Rat Model with PK Data.mod** which accompanies this report.

Measured tissue:plasma partition coefficients for all tissues included in the model were not located for brodifacoum. As an exploratory exercise, the tissue:plasma partition coefficients were estimated using the method of Poulin and Krishnan (1995). This method is implemented in the EXCEL workbook **Partition Coefficients from Poulin and Krishnan 1995.xls** which accompanies this report. The input values needed for this algorithm as well as the estimated values using this algorithm are summarized in Table 2.

The model was optimized using data from Batten and Bratt (1990) on average concentrations in the liver, kidney, and carcass. Liver and kidney data were included because these are excretory organs and data on concentrations of brodifacoum in these organs are available for all three dose levels. Except for the concentrations in the kidney in the low-dose group, the data on liver and kidney included detectable concentrations covering or nearly covering the 104 day duration of the study. Data on concentrations in the carcass are only available for the mid-dose group. These data were included in the optimization because the carcass comprises about 87% of the mass in the model. Thus, even though concentrations in the carcass are low relative to the kidney and liver, this organ was included because it may contain an appreciable fraction of the total body burden.

3. RESULTS

3.1. Model Optimization

The parameter estimates for the PBPK model for brodifacoum are summarized in Table 2 (tissue:blood partition coefficients) and Table 3 (first-order rate constants). Preliminary attempts to fit the model using the parameter estimates from Poulin and Krishnan (1995) were unsuccessful. Subsequently, these partition coefficients were treated as adjustable parameters and were optimized. Taking this approach, the PBPK model provided a reasonable fit to the data ($r^2 = 0.92$, $p = 0.015$).

Figure 2 illustrates the model fit to the observed values at the mid-dose group for concentrations of brodifacoum in liver, kidney, and carcass. Visual inspection suggests that the data are fit reasonably well. Nonetheless, the model does not account for a spike in kidney concentration at Day 1 and the liver concentrations tend to be somewhat underestimated by the model after about 30 days. As illustrated in Figure 3 – liver concentrations for all three dose groups – the underestimates of liver concentration are seen in all three dose groups. In addition, it is apparent that the spike in liver concentrations was not well-modeled using a classical deep compartment. Concentrations in the kidney (Figure 4) are also somewhat underestimated at the high-dose group for the two observations after Day 30. This underestimate is not apparent in the mid-dose group. There are relatively few observations of kidney concentrations in the low-dose group and these are scattered.

4. DISCUSSION

4.1. Model Optimization

The major purpose of this exercise was to determine if the available data on the pharmacokinetics of brodifacoum might be sufficient for the development of a PBPK model that would be useful in assessing potential exposures and risks to target and nontarget animals. The current analysis suggests that the development of such a model is feasible.

This is not to suggest that the current model is adequate or directly useful. It is not. The development of a mature PBPK model would require the inclusion of all data not only from the study by Batten and Bratt (1990) but also from the other studies that are available.

All of the studies will have limitations. The study by Batten and Bratt (1990), for example, involved relatively few and sporadic collections of 24-hour urine and feces samples. This would require an optimization of urine and fecal excretion rates which are generally more difficult to fit than cumulative urinary and fecal excretion data. Similarly, the study in sheep by Lass et al. (1985) provides very little quantitative information on fecal excretion rates and residue data only in carcass, liver, and omental fat. Nonetheless, such data could be used to assess the scalability of a PBPK model developed with data from rats. Species scalability is one of the major benefits in the use of PBPK models and a major criterion in the evaluation of PBPK models (e.g., Bruckner et al. 2004; Durkin et al. 2004). As noted above, data are available on the kinetics of brodifacoum in 16 studies on seven species. Thus, brodifacoum appears to be a compound for which a PBPK model could be developed and well evaluated.

The inclusion of additional data could and probably would lead to a different model than that presented in this report, both in terms of the value of the model parameters and the structure of the model. For example, as noted in Section 2.2, a classical deep compartment was used in an attempt to model the transient peaks noted in the concentrations of brodifacoum in the liver. While this improved the model fit, the improvement was not substantial, the peaks were not well-modeled, and this may have contributed to the underestimates in liver concentrations noted at later time periods at all three doses (Figure 3). This sort of problem is not uncommon during the development of PBPK models and indicates that the model may not be properly structured. Summaries of the study by Parmar et al. (1987) suggest that the pattern in liver concentrations might be better modeled as receptor binding. Explicit receptor binding or the development of empirical models that reflect receptor binding are preferable in PBPK models over the use of classical deep compartments (e.g., Durkin et al. 2004). A receptor binding model component could be considered in a further elaboration of the exploratory model.

4.2. Utility

If a PBPK model were developed and properly validated, it would be useful in assessing several issues associated with the potential risks in the use of brodifacoum for rodent control. It is likely that an at least crude toxicodynamic component could be added. As noted by Batten and Bratt (1990), the coagulation times in rats were associated with concentrations in liver of greater than

about 3 nmol/g tissue. These clotting times returned to normal when liver concentrations dropped below 3 nmol/g tissue but the liver concentrations remained only marginally below toxic levels – i.e., about 1.35 to 2.5 nmol/g liver tissue from Days 8 to 84. Given the slow excretion of brodifacoum and the availability of some multiple dose studies on brodifacoum (Erickson and Urban 2004), it may be possible to model both cumulative effects and the delayed mortality associated with brodifacoum exposures.

Apart from toxicodynamic considerations, the development of a mature PBPK model for brodifacoum could be useful for assessing the potential benefits of different baiting practices. This may be a very practical concern for brodifacoum. As summarized by Erickson and Urban (2004), there is ample data suggesting that brodifacoum will impact both target and nontarget rodents. A PBPK model could be used to assess the potential benefits of different baiting practices in risk mitigation.

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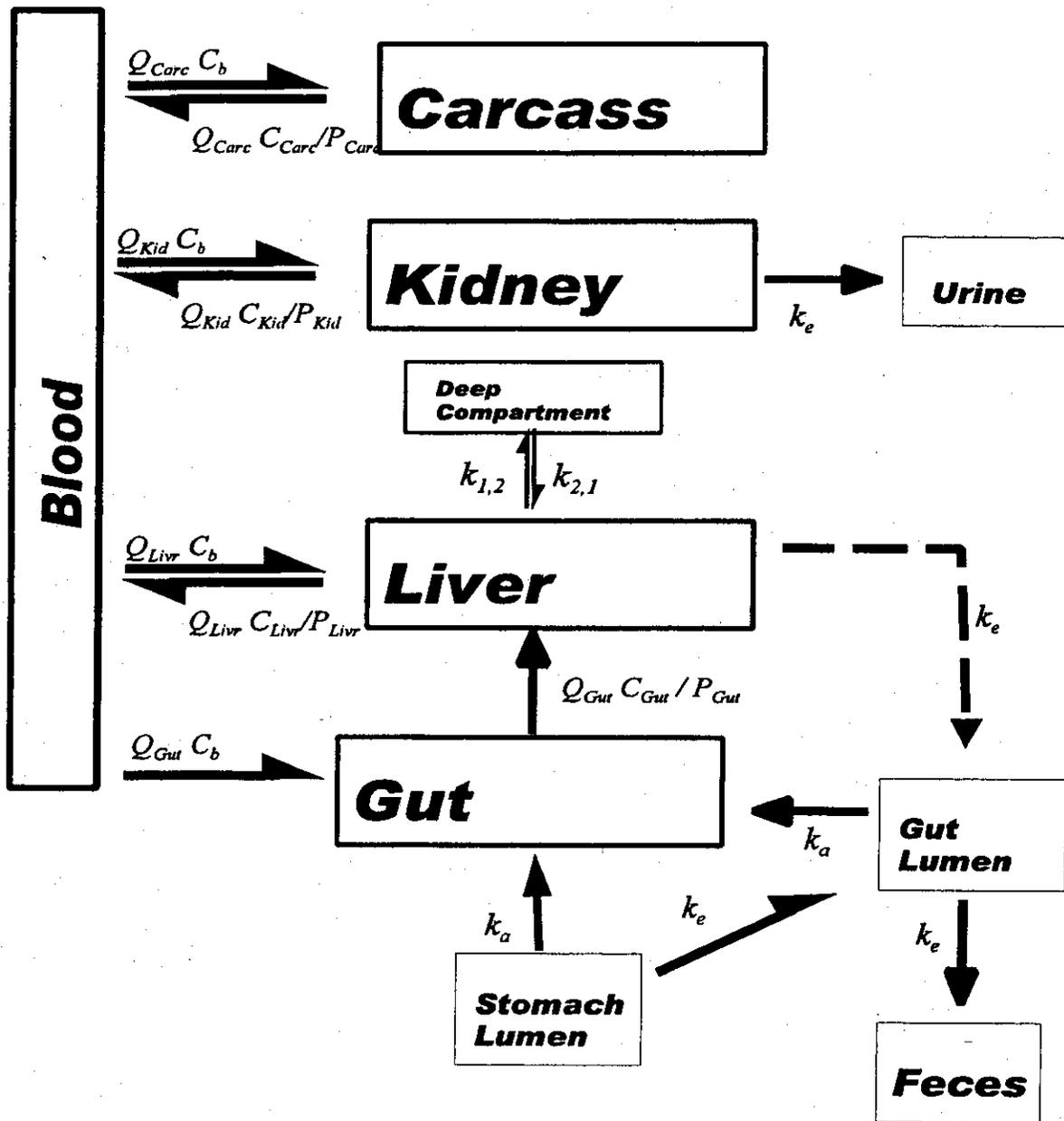


Figure 2: Overview of PBPK Model for Brodifacoum

Figure-1

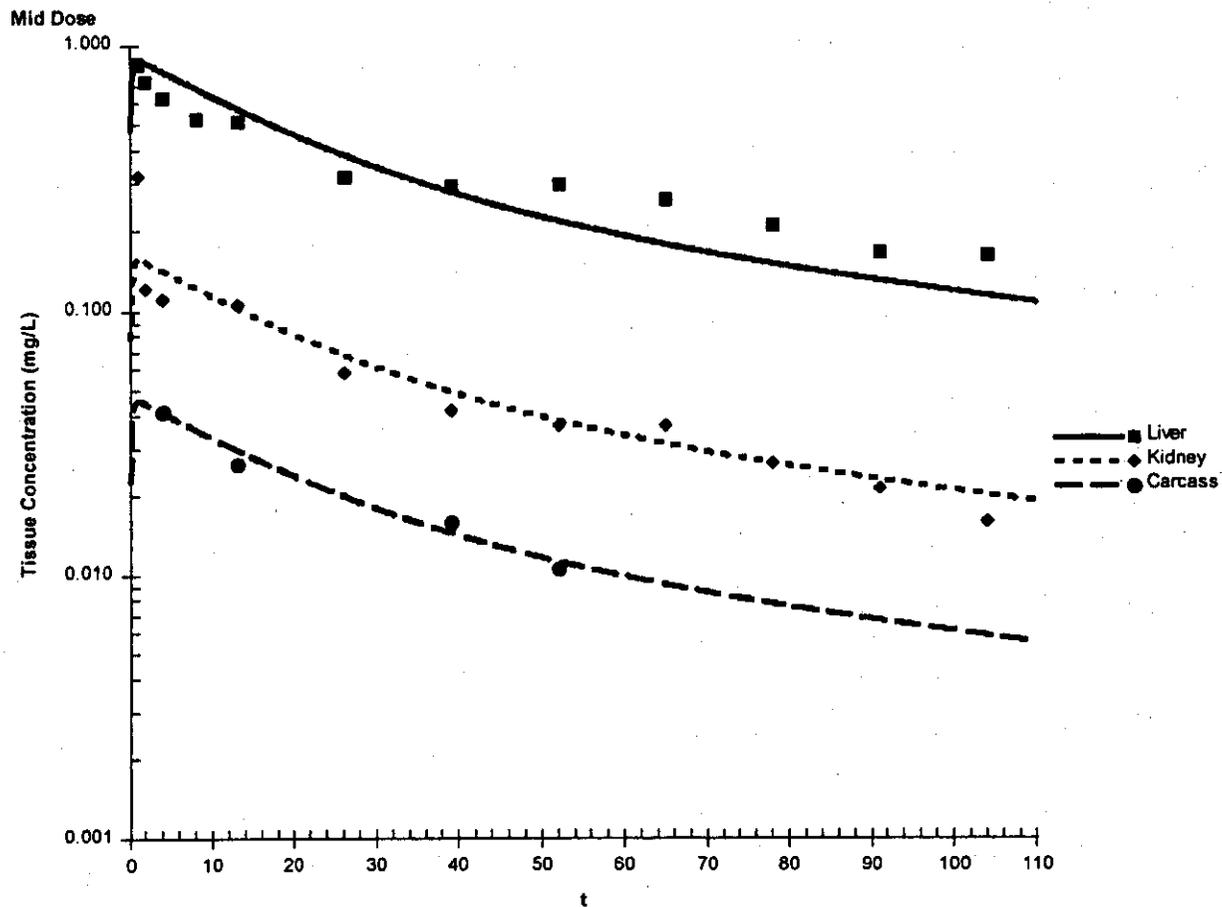


Figure 2: Comparison of observed and modeled concentrations in the liver, kidney, and carcass for the mid-dose group.

Concentration in Liver

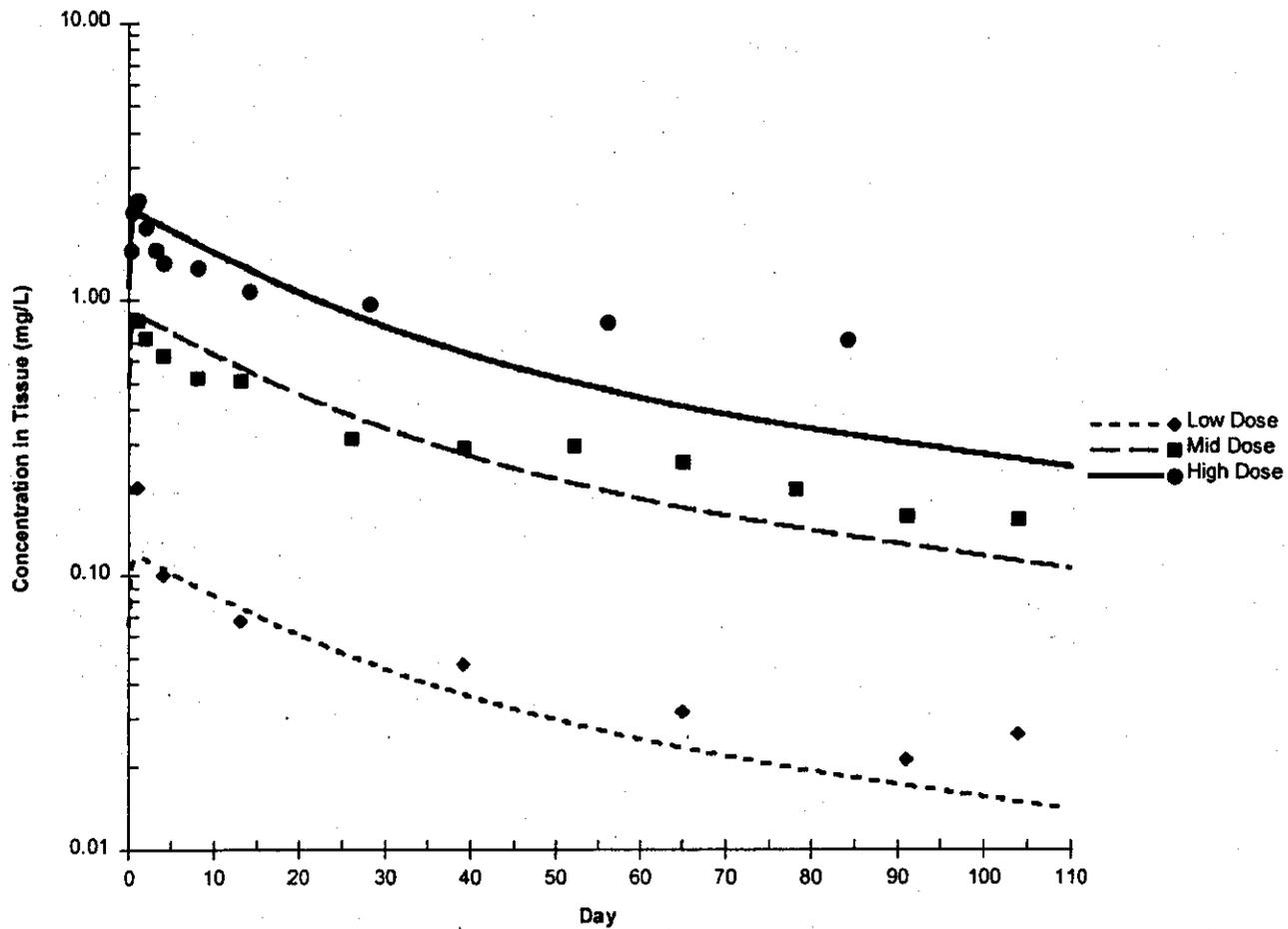


Figure 3: Comparison of observed and modeled concentrations in the liver

Concentration in Kidney

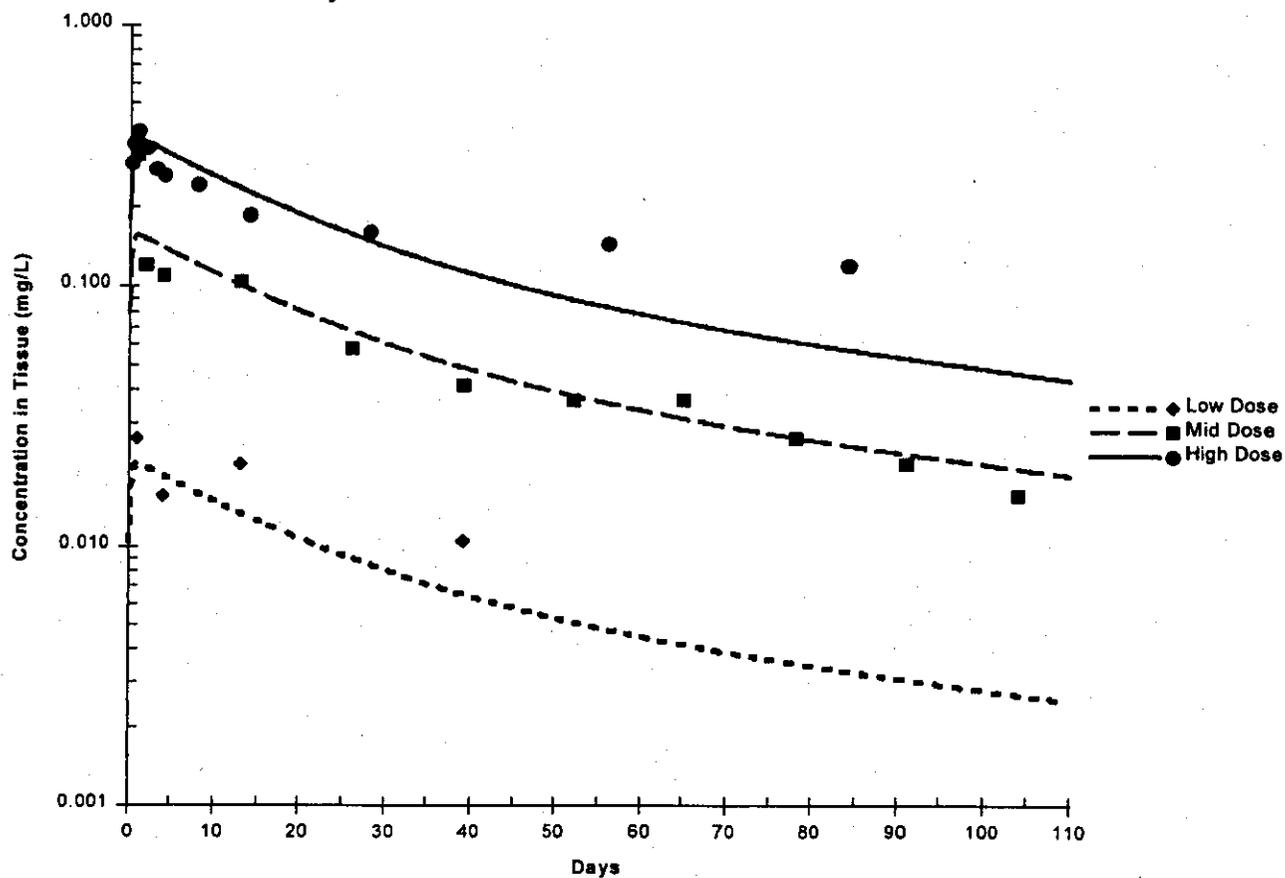


Figure 4: Comparison of observed and modeled concentrations in the kidney

Table 1: Physiological Parameters Used in PBPK Models

Organ	Organ Volume as Proportion of Body Weight ^a		Proportion of Cardiac Output ^a		Units
	Rats	Dogs	Rats	Dogs	
Blood ^b	0.054	0.0720	N/A	N/A	
Carcass	0.8751	0.8528	0.685	0.53	
Gut	0.027	0.0368	0.153	0.251	
Kidney	0.0073	0.0055	0.141	0.173	
Liver	0.0366	0.0329	0.021	0.046	
Other		Rats		Dogs	
Body Weight		0.2		10	kg
Cardiac output ^c		0.235 BW _(kg) ^{0.75}		2.936	L blood/min
		101.2		4227	L blood/day
GI transit (ke) ^d		800 (0.00125)		770 (0.0013)	minutes (min ⁻¹)
		1.8		1.87	day ⁻¹
Stomach transit (ke) ^d		32 (0.03)		96 (0.01)	minutes (min ⁻¹)
		43.2		14.4	day ⁻¹

^a Taken from ILSI (1994) unless otherwise specified.

^b Davies and Morris (1993), Table II, p. 1094

^c Carcass value not specified in ILSI. The value of 0.1 is set judgmentally based on values in other tissue.

^d Davies and Morris (1993), Table III, p. 1094 or Table V, p. 1095. Here and elsewhere, ke signifies first-order excretion rate. The ke values in days⁻¹ calculated as the ke in min⁻¹ × 1440 min/day.

Table 2: Selected Properties of Brodifacoum

Property	Value	Reference	
Molecular weight (g/mole)	523.4249	ChemFinder	
$K_{o/w}$	316,000,000	Epi-Suite	
Tissue:Blood Partition coefficients ($P_{\text{Tissue:Blood}}$)			
	Calculated ¹	Optimized	Units
Liver	21.17 ²	35.8	L/kg
Kidney	18.21	6.4	
Gut	18.24	0.15	
Carcass		1.9	

¹ Calculated from $K_{o/w}$ using the method of Poulin and Krishnan (1995)

Table 3: Estimated first-order rate constants ($r^2 = 0.92, p = 0.015$)

Parameter	Value	Units
Gut k_a	2.4	day ⁻¹
Stomach k_a	11	day ⁻¹
Kidney k_e	1.8	day ⁻¹
Liver k_e	0.13	day ⁻¹
Liver $k_{1,2}$	0.023	day ⁻¹
Liver $k_{2,1}$	0.0000012	day ⁻¹

! Brodifacoum via. ACSL Model Source Code
! PBPK model for brodifacoum in rats.
! Optimized in ModelMaker. See Rat Model v01b Opt 10b.mod
! All time units are in Days

PROGRAM

INITIAL

!Rate constants for deep liver compartment.
CONSTANT Liver_k12 = 0.02034
CONSTANT Liver_k21 = 0.00001279
!Tissue Partition Coefficients
CONSTANT PCarcass = 0.6732
CONSTANT PGut = 0.2174
CONSTANT PKidney = 2.196
CONSTANT PLiver = 14.04
!First order rate constants. These will not change with species.
CONSTANT kaStomach = 0.27048
CONSTANT keKidney = 0.3338
CONSTANT keLiver = 0.2705
CONSTANT kaGut = 0.01729

! ***** Parameters for Rats *****
CONSTANT RatBW = 0.2 !Body Weight, kg
CONSTANT RatQ = 101.2 !Cardiac output, L/day
!The next two parameters are fixed, not optimized.
CONSTANT Rat_keGutLumen = 1.8
CONSTANT Rat_keStomLumen = 43.2
! Blood flow as a proportion of cardiac volume
CONSTANT RatFFCarc = 0.685
CONSTANT RatFFGut = 0.153
CONSTANT RatFFKidn = 0.141
CONSTANT RatFFLivr = 0.021
! Fractional Organ Weights
CONSTANT RatFWBld = 0.054
CONSTANT RatFWCarc = 0.8751
CONSTANT RatFWGut = 0.027
CONSTANT RatFWKidn = 0.0073
CONSTANT RatFWLivr = 0.0366

! Experimental
! High-dose group in rats from Batten and Bratt 1987 for illustration.
CONSTANT RatDoseRate = 0.35 ! mg/kg bw

CONSTANT TSTOP = 110 ! Days
CONSTANT nPoints = 1100 ! Number of points to plot

! Initial Calculated Values
RatDose = RatDoseRate * RatBW
RatBldVol = RatBW*RatFWBld
RatCarcFlow = RatQ*RatFFCarc
RatCarcVol = RatBW*RatFWCarc
RatGutFlow = RatQ*RatFFGut
RatGutVol = RatBW*RatFWGut
RatKidnFlow = RatQ*RatFFKidn
RatKidnVol = RatBW*RatFWKidn
RatLivrFlow = RatQ*RatFFLivr
RatLivrVol = RatBW*RatFWLivr

END ! INITIAL

DYNAMIC

ALGORITHM IALG = 2
CINTERVAL CINT = 0.1

!Use Gear integration algorithm
!Communication interval

DERIVATIVE

```
!***** FLOWS *****  
Gut2Feces = Rat_keGutLumen * RatGutLumen  
RatLivrIn = Liver_k12 * RatLiver  
RatLivrOut = Liver_k21 * LiverDeep  
Lumen2Gut = kaGut * RatGutLumen  
RatBld2Carc = RatCarcFlow * RatBloodConc  
RatBld2Gut = RatGutFlow * RatBloodConc  
RatBld2Kidn = RatKidnFlow * RatBloodConc  
RatBld2Livr = RatLivrFlow * RatBloodConc  
RatCarc2Bld = RatCarcFlow * RatCarcConc / PCarcass  
RatGut2Livr = RatGutFlow * RatGut / PGut  
RatKidn2Bld = RatKidnFlow * RatKidnConc / PKidney  
RatKidn2Urine = keKidney * RatKidney  
RatLivr2Bld = (RatLivrFlow + RatGutFlow) * RatLivrConc / PLiver  
RatLivr2Gut = keLiver * RatLiver  
RatStom2Gut = kaStomach * RatStomachLumen  
RatStom2RatGutLumen = Rat_keStomLumen * RatStomachLumen  
!***** COMPARTMENTS *****  
LiverDeep = INTEG(RatLivrIn - RatLivrOut, 0)  
RatBlood = INTEG(-RatBld2Gut - RatBld2Livr + RatLivr2Bld &  
-RatBld2Kidn + RatKidn2Bld - RatBld2Carc &  
+RatCarc2Bld, 0)  
RatCarcass = INTEG(RatBld2Carc - RatCarc2Bld, 0)  
RatFeces = INTEG(Gut2Feces, 0)  
RatGut = INTEG(RatStom2Gut + Lumen2Gut + RatBld2Gut - RatGut2Livr, 0)  
RatGutLumen = INTEG(-Gut2Feces - Lumen2Gut + RatLivr2Gut + RatStom2RatGutLumen, 0)  
RatKidney = INTEG(-RatKidn2Urine + RatBld2Kidn - RatKidn2Bld, 0)  
RatLiver = INTEG(-RatLivr2Gut + RatGut2Livr + RatBld2Livr &  
-RatLivr2Bld - RatLivrIn + RatLivrOut, 0)  
RatStomachLumen = INTEG(-RatStom2Gut, RatDose)  
RatUrine = INTEG(RatKidn2Urine, 0)  
  
!***** VARIABLES *****  
BodyBurden = RatBlood + RatCarcass + RatGut + RatKidney + RatLiver &  
+RatStomachLumen + RatGutLumen + LiverDeep  
MassBallance = (BodyBurden + RatFeces + RatUrine) / RatBW  
  
! Variables calculated with each integration step.  
RatBloodConc = RatBlood / RatBldVol  
RatCarcConc = RatCarcass / RatCarcVol  
RatGutConc = RatGut / RatGutVol  
RatKidnConc = RatKidney / RatKidnVol  
RatLivrConc = (LiverDeep + RatLiver) / RatLivrVol
```

END ! DERIVATIVE

TERMT (T .GE. TSTOP, 'Model reached TSTOP')

END ! DYNAMIC

TERMINAL

! Unused at this time.

END ! TERMINAL

END ! PROGRAM



**Peer Review of Brodifacoum (PP581) Assessment
WA 2-10**

Submitted to:

**Gail V. Brooks (7507C)
and Gail Maske-Love**

U.S. Environmental Protection Agency
1801 Bell Street
Room 11021-L - Crystal Mall Building #2
Arlington, VA 22202

U.S. EPA Contract No. **68-W-02-035**, WA 2-10
Period of Performance: May 1, 2004 to April 30, 2005 (Option Period 2)
Probabilistic Risk Assessment Support for EFED

Contracting Officer:

Mr. Robert Minjack (3803R)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Ronald Reagan Building
Washington, DC 20460

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January 4, 2005

Table of Contents

1. Introduction	3
Acronyms and Abbreviations	4
2. TECHNICAL ISSUES	5
2.0. General Comments	5
2.1. Conceptual Model	5
2.2. Exposure Assessment	7
2.3. Toxicity Assessment	10
2.4. Risk Characterization	11
Appendix A. Original Comments from Maxine Dakins, PhD	13
Appendix B. Original Comments from Patrick Durkin, PhD	15
Appendix C. Original Comments from Philip E. Goodrum, PhD	33
1. Conceptual Model (Figure 1, p. 77; Section 2.2)	33
2. PT as All or Nothing	34
3. Monte Carlo Analysis	40
Step 1. Outer loop, Interindividual Variability.	40
Step 2. Inner Loop 1, Time Series of Daily Doses	44
Step 3. Inner Loop 1, Calculate Summary Statistics	48
Step 4. Inner Loop 2, Time Series of Cumulative Doses (Body Burdens)	49
Step 5. Inner Loop 2, Summary Statistics	49
Appendix D. Original Comments from Maria Mastriano, MS	55

ATTACHMENTS

Attachment 1: A Physiologically Based Pharmacokinetic Model for Brodifacoum

1. Introduction

U.S. EPA requested a peer review of the ecological risk assessment on Brodifacoum submitted by Syngenta Crop Protection, Inc. in September 2004. This review focuses primarily on the scientific merits of the analysis. In order to better understand how the analysis was conducted, we referred to electronic files provided with the report (summarized below). These files were used to both rerun selected analyses, and to modify the models to more fully explore the consequences of the assumptions presented by the report authors. We did not attempt to perform a full QA/QC of all of the steps in the analysis.

This report synthesizes all of the comments into general issues, as well as issues specific to the conceptual model, exposure assessment, toxicity assessment, and risk characterization. Detailed comments from each individual are included in Appendices. The following questions posed by Mary Frankenberry helped to guide our review:

1. Are the assumptions in the analysis well supported?
2. If alternative assumptions could have been made, what were the consequences of the approaches used on the overall risk estimates? Did the approaches used tend to increase or decrease risk?
3. Should additional questions be posed, or should additional materials be requested of Syngenta, in order to improve the clarity and transparency of the analysis?

The following materials were submitted to the Agency and were considered in this review:

1. Risk assessment report entitled, "A Probabilistic Assessment of the Risk of Brodifacoum to Non-target Predators and Scavengers. Prepared by The Cadmus Group, Inc., Jeffrey Giddings and William Warren-Hicks, on behalf of Syngenta Crop Protection. September 10, 2004. Syngenta Number T001270-04." Hereafter, this reported is referenced as Cadmus (2004). The file name is 1270-04 Assessment Publication.pdf.
2. Conceptual Model Overview - MS Power Point files
 - EPA Model Demo 06Oct04.ppt
 - Wash talk February 2004.ppt
3. Monte Carlo Analysis - MS Excel files
 - CD001-01.xls
 - DD001.xls
 - Exposure Runs.xls
 - GHO_CD001-001.xls
 - GHO_DD001.xls
 - KF_CD001-001.xls
 - KF_DD001.xls

4. Bayesian Analysis - Winbugs analysis files
 - winbugs input-bird data-FINAL.txt
 - winbugs input-mammal data-FINAL.txt

5. SAS files
 - bird dose-response plot FINAL.sas
 - final risk code.sas
 - generate bird data - FINAL.sas
 - generate mammal data - FINAL.sas
 - mammal-response plot FINAL.sas

It should be noted that these files were sufficient to evaluate some, but not all of the analyses presented. Specifically, only selected Excel files were provided to rerun Crystal Ball simulations to reconstruct the exposure assessment. For example, the file CD001-01.xls provided selected summary statistics from the 2-D Monte Carlo simulation for coyote; runs for other receptors were not available. The SAS files provide some insights into how the exposure and toxicity distributions were combined, however, specific files that are referenced in input file commands were not provided. Therefore, the risk calculations could not be easily reproduced.

The references cited in the Cadmus report should have been submitted with the document. Some key references were obtained during the review and these references were useful. If additional citations had been readily available, they would have made the review process more efficient and the quality of the review would have been enhanced.

A number of acronyms and abbreviations appear in the Cadmus report and in our comments. The following list of the more frequently used terms is provided here for convenience.

Acronyms and Abbreviations

PD	=	% rodents in diet
PT	=	% rodents exposed to rodenticide
FIR	=	food ingestion rate
log(a), b	=	allometric scaling factors
Wt	=	body weight
GE	=	gross energy content of food
AE	=	assimilation efficiency
M	=	moisture content of food
FMR	=	field metabolic rate (function of log(a), b, and Wt)
DPD	=	daily PD
DPT	=	daily PT
C	=	concentration in exposed rodents
t _{1/2}	=	halftime for first order elimination kinetics

410

2. TECHNICAL ISSUES

This section summarizes the main comments on the brodifacoum risk assessment. Detailed comments from each of the reviewers are included as appendices.

2.0. General Comments

The overall approach used to characterize risks to birds and mammals reflects an attempt to develop refined estimates of risk by incorporating variability and uncertainty in exposure and dose response. The objective is commendable and has the potential to provide useful information for the risk management of brodifacoum and other rodenticides. However, the analysis presented by Cadmus (2004) is an incomplete and superficial assessment that ignores relevant data, is based on an inappropriate pharmacokinetic model, and includes questionable assumptions that, in most cases, can be expected to substantially underestimate exposure and risk. The conclusions reached in the Cadmus (2004) submission are of little use in assessing risks posed by brodifacoum used in effective rodent control programs.

Key shortcomings of the analysis are identified in the following sections. Table 1 summarizes the consequences of the approach that was used in terms of the potential impact on the overall risk estimate. This effect is characterized qualitatively (i.e., an alternative approach would tend to yield higher or lower estimates of risk). In cases where we demonstrate an alternative assumption or modeling approach, the effect on the risk estimate is expressed quantitatively in the more detailed comments. Table 1 also serves as a cross reference to specific comments in each Appendix.

The results of the analysis lead the authors of the Cadmus report to conclude that risks to terrestrial receptors are low, and unlikely to exceed levels of concern for acute effects. This conclusion seems to be based on a large number of unsupported assertions all of which lead to lower estimates of risk. These sort of broad and unsupported statements detract from the sense of objectivity that a risk assessment should have. The basic assumption appears to be that the exposure of rodents to the rodenticide is negligible to low ($PT=0.01$ to 0.15). Thus, the risks to other organisms consuming the rodents will probably be negligible to low as well. This conclusion, however, is of little use in assessing risks posed by brodifacoum used in effective rodent control programs.

2.1. Conceptual Model

1. The conceptual model excludes potential routes of exposure and does not carry forward several of the pathways that were identified.
 - a. Direct ingestion of bait by predators/scavengers is identified as a potential (primary) route of exposure, but it is not included in the assessment. A relatively simple exposure model can be used to address this exposure pathway (see Appendix B, Comment 2; Appendix D, Figure 1).

- b. The conceptual model identifies rodents as the target species for baiting. All nontarget animals other than rodents (e.g., rabbits, birds, larger mammals, etc) are apparently excluded. However, these are also potential prey species for at least some of the predators considered in the assessment. For example, several studies demonstrate that rabbits comprise more than 25% (by volume) of the stomach contents of red fox (see Appendix D). Furthermore, these are species that could easily consume the bait directly, or via diet. For example, coyote may be a predator of both birds and fox. Also, it is unclear if the information used to define PD is intended to be inclusive of all small rodents (e.g., mice, voles, squirrels), or if it is specific to rats. To the extent possible, all target and nontarget prey species (feeders and carcasses) should be included.
 - c. Invertebrates such as beetles and ants that are consumed by some of the predators are excluded from the dietary model as a source of brodifacoum exposure. Since invertebrates do comprise a non-negligible portion of the total diet, especially during the summer, we question whether this is truly a minor source of uncertainty in the overall exposure model. The major uncertainty stems not so much from the estimate of the percent of diet comprised of invertebrates ("PD_invertebrates"), but rather from the lack of information available to determine the fraction of invertebrates that are consumed and contaminated (i.e., "PT_invertebrates"). This PT term likely varies as a function of baiting practice. It could easily be explored in a series of alternative, plausible scenarios, similar to what was done with the PT term for rodents.
2. The assessment does not adequately describe the underlying assumptions concerning how the bait is used and applied and does not address uncertainties associated with baiting practices.
 - a. The assessment attempts to model exposure under the assumption that baiting practices are performed according to the directions on the label. The use characterization should be more fully described. Very different exposure scenarios may be reflected by the assumptions regarding baiting. A successful bait scenario would result in a higher number of carcasses over a short period of time. A less successful bait scenario would result in a lower number of carcasses in the short term, but possibly a prolonged period of exposure for predators.
 - b. It was assumed the amount of product used was consistent with the directions on the label. This assumption is questionable and should be explored through sensitivity analysis.
 - c. It was assumed that indoor use would not lead to an increase in exposure to nontarget species. This assumption is questionable and should be explored through sensitivity analysis.

2.2. Exposure Assessment

1. The selection of PT (the proportion of rodents exposed to brodifacoum) appears to be arbitrary, incorrect, and biased.
 - a. PT is varied across a vary narrow range, with a maximum of 15%. This is unlikely to reflect the extent of contamination associated with successful baiting practices. The full range (up to PT = 100%) should be illustrated, especially since this variable is defined exclusively based on judgement rather than available data.
 - b. As used in the Cadmus report, PT appears to be a composite parameter reflecting exposures to both target rodents as well as nontarget organisms. It is likely that the analysis would have been clearer if these two factors were explicitly distinguished. Field data are available indicating that PT can exceed 80% for target rodents. The Cadmus report appears to assume that PT for nontarget rodents is much lower and that the consumption of target rodents will be low. The report, however, does not adequately discuss or justify these assumptions.
 - c. The Cadmus report states that: *The proportion of Norway rats and house mice that are exposed to a brodifacoum baiting program is unknown, but is likely to be much smaller than one in ten* (Cadmus, 2004, p. 27 of 114). The value of one in ten is unsupported. This seems to explicitly postulate an ineffective baiting program – i.e., only a small proportion of the target organisms will be exposed.
 - d. The argument in the Cadmus report that the true PT value in the field is at or below 1% seems without merit and at very least is open to debate. This assumption could be construed to be serving the interests of a particular group.
2. The selection of values for PD (proportion of rodents in the diet of predators) and the estimates of the variability of PD appears to be overly restrictive.
 - a. The use of only **PD** (proportion of rodents in the diet) as an input for dose ignores both direct consumption of the bait by nontarget species as well as the consumption of other types of animals (e.g., birds and insects) that may contain toxicologically significant amounts of brodifacoum. The importance of both direct consumption of bait and the consumption on non-rodent organisms is amply demonstrated
 - b. The variability in daily percent diet from rodents (PD) is represented by a mean PD and an assumed standard deviation. The parameters are assumed to be independent, suggesting that individuals with very low percentages of rodents in diet exhibit the same variability in diet as individuals with very high percentages of rodents. This seems like a less plausible assumption than the use of a fixed CV, which ensures that higher mean PD is also associated with higher variability in PD.

3. The probabilistic approach does not address potential sources of variability and uncertainty.
 - a. PT is modeled as all or nothing for any given day. An alternative, and perhaps more plausible scenario is that PT should be applied to individual prey meals. The result will be a time course of more frequent daily doses instead of the long periods of no exposure that are currently simulated when PT is set to a low value (e.g., 3%).
 - b. The correlation between the log(a) and b parameters of the FMR equation is ignored. This is a source of uncertainty that could result in an underestimate of the 95th percentile FMR by as much as 10%.
 - c. Total daily consumption is modeled instead of individual prey meals. This does not account for gorging behavior (i.e., exceedance of average daily FIR), which is likely for opportunistic feeders like the fox and coyote. A simple prey meal consumption scenario could be incorporated into the daily dose inner loop by estimating the body weight of each prey item consumed. Data on common prey items such as rats, mice, meadow voles, and rabbits are readily available.
 - d. A lognormal distribution is used to describe variability in DPD. Alternative plausible probability distributions should be explored as part of the uncertainty analysis, especially if DPD is identified from a sensitivity analysis as a major source of variability in the model output (i.e., distribution of daily body burdens).
 - e. Seasonal variability in dietary habits will result in periods of the year in which rodents comprise a greater percentage of the diet. Data on seasonal variability should be taken into consideration in defining the appropriate input distribution for PD.
 - f. Variability in exposure and effects is expected as a function of developmental status. Risks to kits, for example, may be greater than risk to adult foxes.
4. The reliance on monitored concentrations of brodifacoum in rat carcasses limits the ability of the analysis to reflect variations in baiting practices and may underestimate risk.
 - a. The Cadmus report states that: *Earlier drafts of the analysis plan had assumed that submodels would need to be developed to estimate concentrations in food.* This would have been preferable to the approach taken in the document and would have allowed the analysis to assess differing risks among different baiting practices. The modeling effort could have been validated with the monitoring data that are available with specific baiting practices.
 - b. The reliance on monitoring data may have modestly simplified the analysis but this approach ignores temporal relationships that may lead to an underestimate of risk. In other words, the number of rodents (target and nontarget) that are exposed to

brodifacoum and the concentrations of brodifacoum in these organisms is likely to vary over time. The failure to consider this may miss periods of peak exposure in predators.

5. The simulation approach focuses on summary statistics that are not conservative.

The first inner loop is used to develop a sequence of daily doses for each of 100 individuals. Each sequence is then fit to a lognormal distribution defined by a mean and standard deviation. Then, the parameters of the lognormal are characterized by distributions - a beta is used to represent variability in the mean daily dose, and a gamma is used to represent the coefficient of variation (CV) daily dose. Next, a set of parameters (mean, SD = CV/mean) is determined, and a new time course of daily doses is simulated to estimate daily body burdens of brodifacoum.

- a. The first summary statistic calculated is the maximum body burden during the first 90-day period for one individual. Depending on the choice for the half-life value used to represent elimination and metabolism, bioaccumulation may occur such that body burdens associated with the first 90-day window of exposure will be much lower than body burdens later in the year. The maximum 1-year body burden would be a more appropriate metric than the maximum of the first 90 days.
 - b. The second summary statistic calculated is the arithmetic mean of the maximum 90-day body burdens estimated from 100 consecutive simulations of the individual described above. This could be interpreted as either an alternative realization of the dosing for one individual, or an attempt to model 100 different individuals with the same general daily patterns. This step is unnecessary, and not protective. It effectively dampens the variability in peak exposures. Instead, each individual simulation should be used to generate a plausible maximum.
 - c. The third summary statistic involves generating an empirical probability distribution based on 100 separate estimates of the mean of the maximum 90-day body burdens described in (b) above. It is unclear why only 100 individuals are simulated, and there was no attempt to demonstrate that 100 individuals is sufficient to achieve stability in the characterization of the upper tail (> 90th percentiles) of the distribution.
6. The justification for and documentation of the distributions used in the Monte Carlo simulation are incomplete. For example, probability distributions used in the analysis are not fully described in the text. At a minimum, the following information should be given: the type of probability distribution, the parameter values, summary statistics if the parameter values are not obvious to most readers, and a graphic (probability density function and/or cumulative distribution function).
- a. All choices of probability distributions should be justified or at least discussed and summarized in a table and with graphics.

- b. It is unclear if unbounded distributions like the lognormal and gamma, which are used in this analysis, are actually truncated to reflect the constraints of the variable. For example, the lognormal PDF for percent diet comprised of rodents should be truncated at 100%.

2.3. Toxicity Assessment

1. Use of the full dose-response curve in the risk calculation is an improvement over the use of species sensitivity distributions. However, some of the methods and results associated with the aggregation of toxicity data need further explanation.
 - a. Definitions of sensitive species is subjective. It is unclear why a sensitive species was defined as the 10th percentile and not the 5th percentile or 2.5th percentile. Defining the bounds as the 5th and 95th would lead to 90% confidence while using the 2.5th and 97.5th would lead to a 95% confidence of including the dose response curves of nontarget species. A 80% confidence seems inappropriately low in this instance.
 - b. The bounds shown in Figures 15 and 16 do not appear to represent the variability that exists, even within these very limited data sets, and should not be used to define what the dose-response relationship would be for a hypothetical sensitive nontarget species. The bounds do not include even 80% of the dose-response curves used. For birds, out of 9 curves, only 3 are completely contained within the 80% confidence bounds. For mammals, one curve lies left of the lower bound, two are partially left of it, two are completely right of the upper bound, and two are partially right of it. Of the 8 curves shown, only 1 is completely contained within the 80% bounds.
 - c. For the Bayesian approach, discussion and justification should be provided for the assumption that both alpha and beta are normally distributed. If it was simply done for convenience, it would be helpful to have a sensitivity analysis on that assumption. This is particularly important given that the 80% confidence bounds on the dose/response curves did not appear to cover 80% of the curves.
 - d. The Cadmus report should justify or at least discuss the basis for using the logistic transformation (over the more commonly used probit transformation) in the dose-response assessment.
2. The discussion and application of the single compartment kinetic model is based on a superficial and incomplete review of the available data and an incorrect assessment of the available data on the pharmacokinetics of brodifacoum. The use of the single compartment kinetic model is unnecessary, inappropriate, and limits the assessment of the adverse effects that may occur. (See detailed comments in Appendix B.)

- a. Information on the pharmacokinetics of brodifacoum are available in 16 studies on seven species. None of this information is meaningfully discussed in the Cadmus document.
- b. Cadmus (2004) report states that there is a *pool of rapidly eliminated brodifacoum* (Cadmus, 2004, p. 21 of 114, lines 1 to 2). This appears to be a misinterpretation of the classical two compartment model. There is a biphasic pattern in the concentration of a compound of brodifacoum. The first and more rapid phase is associated with redistribution, not excretion.
- c. In the same paragraph, the Cadmus report states that *...the toxicokinetics of brodifacoum, particularly the rates of exchange between blood, liver, and other tissues, have not been quantified even in the rat*. This is also largely incorrect and certainly misleading. One of the 16 available studies (Batten and Bratt 1990) was consulted in the current review. The study appears to be reasonably well done and is not difficult to interpret.
- d. Also in the same paragraph, the Cadmus (2004) report states that *many mechanistic and quantitative assumptions* would be need to model the kinetics of brodifacoum. This is also incorrect. As illustrated in Attachment 1, a reasonable albeit exploratory PBPK model can be developed using only the residue data from Batten and Bratt (1990). The kinetics of brodifacoum can be described by a simple flow-limited PBPK model that can be optimized with the concentration data in liver, kidney, and carcass (which account for the bulk of the brodifacoum in the rat).
- e. The Cadmus (2004) report chooses a range of halftimes from 2 days to 200 days with the implication that the "real" halftimes are somewhere in this region. In terms of the body burden of the prey species, this is not correct. The shorter halftimes of 2 days to several days reflect the blood data and redistribution from blood to tissue. This has nothing to do with body burden. The upper range of 200 days is presumably based on liver halftimes. As reviewed by Erickson and Urban (2004), reported liver halftimes are in the range of about 74 to 350 days.
- f. The same paragraph goes on to discuss the use of the first order model and a sensitivity analyses using a range of values. The value of sensitivity analyses on a structurally incorrect model is minimal and leads to the mis-impression that the kinetics have minimal impact on the assessment of risk.

2.4. Risk Characterization

1. The approach to risk characterization taken in the Cadmus report involves comparing single dose gavage LD₅₀ doses to peak body burdens from intermittent dietary exposures over a prolonged period – e.g., 90 days in most of the simulations conducted by Cadmus (2004). The underlying assumption in this approach is that toxicity is based on peak body

burden. This assumption should be justified. The Cadmus report should have discussed and possibly considered the use of time-weighted average (TWA) body burdens (e.g., Haber's Law) rather than peak body burdens. This is important for intermittent exposures to slowly eliminated compounds.

2. The Cadmus (2004) report should have attempted to reconcile their analysis with the rather clear and compelling body of experience with brodifacoum reflected in field studies that clearly indicates that risks to nontarget species are not only plausible but are well-documented. An attempt to use field data as a check of conclusions should always be part of a PRA, particularly one in which many of the assumptions are not well-supported.
3. The risk estimates appear to be based on a distribution from the exposure assessment that reflects the mean 90-day maximums across 100 individuals, rather than all 10,000 estimates of the 90-day maxima that were calculated. If true, the approach used could greatly underestimate the upper tail ($> 90^{\text{th}}$ percentile) of the risk distribution. An example given in Appendix C (Comment 15) illustrates the difference between the two approaches. The approach using the mean 90-day maxima yields a distribution with a 40% lower dose at the 5% exceedance level.
4. The sensitivity analysis of the probabilistic model is incomplete. The uncertainty analysis does not adequately address alternative, plausible modeling approaches and input assumptions.
5. A Tier 1 analysis (point estimate approach) is not presented along with the probabilistic analysis.

Appendix A. Original Comments from Maxine Dakins, PhD

1. They laid out a conceptual model in Figure 1 but did not include all possible pathways of exposure and they did not carry forward several of the pathways they did identify.
 - a. Specifically, they identify Nontarget Bait Feeders and Nontarget Carcasses as important components of the conceptual model but exclude them in the calculations.
 - b. They did not include, in concept or in the calculations, the pathway of Direct Ingestion of Bait by nontarget organisms.
 - c. In addition, they made assumptions regarding use that affected their parameterization of their model. Specifically, they assumed the product was always distributed according to the directions on the label (limiting direct outdoor exposure to bait) and they assumed that indoor use would not lead to an increase in exposure to nontarget species. Neither of these assumptions seems to me to be beyond debate. They should, at the least, be explored through sensitivity analysis.
2. Their use of a 90-day period for simulation seems insufficient. The authors state on page 46 that "Extending the simulation for longer periods (180 or 360 d) resulted in a 2- to 3-fold increase in maximum cumulative exposure". Given this, it is unclear why the authors decided not to run all simulations for 360 days or longer. In addition, it is unclear why the authors "did not carry these exposure estimates forward to determine the effect of the simulation period on risk".
3. Their exploration of the percentage of exposed rodents in the diet (PT) through sensitivity analysis seems appropriate given the lack of data.
 - a. However, their limitation of this variable to less than 15% appears to be without justification. In the absence of limiting data, the sensitivity analysis of PT should include a full range of values up to 100%. The authors state "In the immediate vicinity of a structure where baiting is in progress, PT may approach 1".
 - b. They present a sensitivity analysis of this variable only for coyote which is the fourth most sensitive to brodifacoum of the five surrogate non-target species they explore. The results of their analysis for coyote (Figure 24) do not even include their highest value of PT=0.15. A full sensitivity analysis should be performed and included for all surrogate non-target species, particularly those known to be sensitive to brodifacoum, such as the great horned owl and kit fox.
 - c. Finally, their argument that the true PT value in the field is at or below 1% seems without merit and open to debate. This assumption could be construed to be serving the interests of a particular group.
4. In the effects analysis, their use of Bayesian hierarchical modeling is interesting and innovative. This analysis, unlike the species sensitivity distribution approach, should lead to an entire estimated dose-response curve for a hypothetical sensitive non-target species. However, the execution of this approach here raises some concerns.

- a. It is unclear why a sensitive species was defined as the 10th percentile and not the 5th percentile or 2.5th percentile. Defining the bounds as the 5th and 95th would lead to 90% confidence while using the 2.5th and 97.5th would lead to a 95% confidence of including the dose response curves of nontarget species. A 80% confidence seems inappropriately low in this instance.
 - b. Depending on the applicability of the species where dose response data exists to the species likely exposed in the field, a conservative approach should be used and carried out through the definition of a high confidence level in a)
 - c. Most troubling, the bounds shown in Figures 15 and 16 do not appear to include even 80% of the dose-response curves used. For example, for birds, out of 8 dose response curves (ignoring the 1986 pheasant data), two curves lie left of the lower bound and three lie partially right the upper bound. The pheasant curve violates both bounds. Therefore, out of 9 curves, only 3 are completely contained within the 80% confidence bounds. For mammals, one curve lies left of the lower bound, two are partially left of it, two are completely right of the upper bound, and two are partially right of it. Of the 8 curves shown, only 1 is completely contained within the 80% bounds. These bounds, as shown, do not appear to represent the variability in dose response curves that exist, even within these very limited data sets, and should not be used to define what the dose-response relationship would be for a hypothetical sensitive nontarget species.
 - d. For the Bayesian approach, they assume that both alpha and beta are normally distributed without any discussion and/or justification of this assumption. It might be a reasonable assumption but I would like to hear their justification for it. If it was simply done for convenience, it would be helpful to have a sensitivity analysis on that assumption. This is particularly important given that their 80% confidence bounds on the dose/response curves did not appear to cover 80% of the curves.
5. As far as information EPA should request from Cadmus, I believe they should ask for the full sensitivity analysis on the variable PT for all levels and all species. I would guess Cadmus did all of them but there's no guarantee. They don't even report all levels for Coyote (as noted in 3b above).
 6. As far as studies/data, they report none on the pathways that they disregard. So, for Direct Ingestion of Bait, what is the literature? Also for Ingestion of Nontarget Species and Carcasses? They decide to eliminate these pathways without appropriate justification, certainly without discussing the literature.

Appendix B. Original Comments from Patrick Durkin, PhD

I have reviewed the report by Cadmus (2004). I have focused my review on how the available information on the pharmacokinetics and toxicokinetics of brodifacoum are incorporated (or not incorporated) into the analysis presented by Cadmus (2004).

Most specific comments are identified by page and paragraph number (e.g., p. 1, ¶2 for page 1, paragraph 2). The Cadmus (2004) document has two differing sets of page numbers, one at the top of each page and another at the bottom. All page numbers cited below refer to the page number specified on the bottom of the page. Paragraphs are counted from the top of each page with any initial partial paragraph designated as ¶1. Line numbers are identified only when necessary and always refer to the line in the paragraph that is being referenced. Quotations from the Cadmus (2004) report are given in italics as needed to clarify comments.

In any draft that is being sent out for external review, the use of line numbering should be considered.

p. 12, ¶2 and 3

These are the only paragraphs that mention the large number of incident data that are available on brodifacoum. The report goes on to present an analysis suggesting no substantial risks to nontarget species. The analysis should have attempted to reconcile the incident data summarized by Erickson and Urban (2004) as well as incident data reported in publications cited in the Cadmus (2004) report (e.g., Edwards and Swaine 1983; Edwards et al. 1983a,b).

p. 13, ¶3 to 5

Mortality is used as the sole endpoint of concern. The estimates of mortality are based on standard laboratory studies. As discussed by Erickson and Urban (2004), there is some evidence that wildlife may be at greater risk because of the possibility that exposures to brodifacoum at doses that are sublethal in laboratory studies could cause adverse effects (including mortality) in wildlife secondary to injuries (e.g., bruising) that would otherwise be sublethal. This is acknowledged in the Cadmus (2004) report:

Lethal poisoning, as well as direct sublethal effects that could increase the likelihood of individual mortality (e.g., hemorrhaging), are of greatest concern.

P. 13, ¶5, line 3 ff

The analysis by Cadmus (2004), however, focuses solely on lethality and does not present a consideration of the sublethal adverse effects that could be associated with hemorrhaging in wildlife.

p. 14, Last ¶

While not a serious issue, general statements asserting that water and air concentrations are unimportant are not well supported. While I do not think that either water or air concentrations

are important relative to dietary intake, an at least preliminary exposure assessment to demonstrate this would have been appropriate.

p. 15, ¶ 1

This paragraph is filled with assertions that are unsupported and appear to be unsupportable. The statement that *secondary exposure to predators and scavengers appears to be of greater concern to regulators than risk of primary exposure* simply does not make any sense and I doubt that it reflects the position of EFED. Similarly, the statement that a *fundamentally different exposure model* would be required is incorrect. The statement that invertebrate feeders are considered unimportant is not supported and does not appear to be supportable. The significance of invertebrate bait feeders to birds is demonstrated by incident data (see Thorsen et al. 2000 as cited in Erickson and Urban 2004, p. 65). These sort of broad and unsupported statements detract from the sense of objectivity that a risk assessment should have.

p. 15, ¶ 4, last sentence

The decision to use monitoring rather than modeling may underestimate risk and perhaps substantially. This ignores temporal relationships in brodifacoum concentrations in prey that are plausible. The implication that *complex behavioral simulations* would be needed is incorrect. Relatively simple models could be employed that might lead to more credible assessments of risk.

p. 15, Last ¶

While a relatively minor point, the Cadmus (2004) report repeatedly uses the phrase *cumulative dose* – which in common usage would be the total of all the doses that are consumed over some period of time – when they appear to be referring to *body burden* – the total amount of the chemical in the organism at any given time.

p. 15, ¶ 1 and 2

The discussion of Kaukeinen et al. (2000) is unclear. What other toxic effects are caused? The liver binding with consequent interference in clotting factors appears to be the mechanism of concern.

p. 18 and elsewhere

Use either *non-target* or *nontarget* consistently. The latter is preferable.

p. 19, ¶ 4

Red-tailed hawks will prey on other birds (e.g., morning doves and starlings) which could be exposed to bait either directly or through the consumption of insects. Some of the studies in the source cited in the Cadmus (2004) report (e.g., U.S. EPA 1993) indicate that birds may comprise 10% or more of the hawks diet (see Volume II of U.S. EPA 1993, pp. A-91 ff). Ignoring this source of exposure will bias the analysis to an underestimate of risk. Other paragraphs on this and the following page do talk about the consumption of invertebrates and birds but this is not addressed in the model.

p. 20, ¶ 3

Earlier drafts of the analysis plan had assumed that submodels would need to be developed to estimate concentrations in food. This would have been preferable to the approach taken in the document. The reliance on monitoring data may have modestly simplified the analysis. This approach, however, ignores temporal relationships that may lead to an underestimate of risk. In other words, the number of rodents (target and nontarget) that are exposed to brodifacoum and the concentrations of brodifacoum in these organisms is likely to vary over time. The failure to consider this may miss periods of peak exposure in predators.

p. 20, ¶ 4

I understand what *PT* is supposed to be but I do not see here or elsewhere how *PT* is set in any objective way.

p. 20, ¶ 5

The use of the maximum concentration or maximum body burden should be justified in some way. For brodifacoum, it is plausible that concentrations in the liver could be a more reasonable measure of exposures that would be toxicologically significant. Also, the analysis would be enhanced by considering or at least discussing the issue of delayed death.

p. 20, Last ¶ to p. 21 ¶1

Depuration kinetics of brodifacoum are complex, as described in Sections 2.2.3 and 3.3.2.2. Studies in rats suggest that depuration follows a biphasic curve (Kaukeinen et al. 2000). The initial phase of the curve is steep, with a half-life on the order of a few days, reflecting a pool of rapidly eliminated brodifacoum in the body. The second phase of the depuration curve is much slower, with a half-life on the order of many months, reflecting a much smaller pool of tightly bound brodifacoum in the liver. However, the toxicokinetics of brodifacoum, particularly the rates of exchange between blood, liver, and other tissues, have not been quantified even in the rat. Many mechanistic and quantitative assumptions would be needed to simulate the biphasic depuration process, and the results would reflect large uncertainties in these assumptions. ...

This is a very important paragraph and virtually nothing in this paragraph is correct.

Neither Section 2.2.3 nor 3.3.2.2 describe kinetics (complex or simple) in any meaningful way. Information on the pharmacokinetics of brodifacoum are available in 16 studies on seven species: rats (Bachmann and Sullivan 1983; Batten and Bratt 1990; Belleville 1991; Bratt and Hudson 1979; Hawkins 1991; Parmar et al. 1987), possum (Eason et al. 1996), rabbits (Breckenridge et al. 1985), sheep (Lass et al. 1985), dogs (Robben et al. 1998; Woody et al. 1992), horses (Boermans et al. 1991; McConnico et al. 1997) and humans (Bruno et al. 2000; Hollinger and Pastoor 1993; Weitzel et al. 1990). Additional information may also be available in Eason et al. 2002. None of these studies are mentioned in the discussion of the purportedly complex kinetics. The one reference that is cited (Kaukeinen et al. 2000) is from a conference proceeding on risk

benefit considerations of anticoagulants. While this paper was not available for the current review, it seems reasonable to assume that the paper does not involve a critical review of kinetic studies on brodifacoum.

Given to the failure of the document to clearly describe the kinetic data on brodifacoum, the reviews by Erickson and Urban (2004) and WHO (1995) were consulted and the study by Batten and Bratt (1990) was examined. The statement by Cadmus (2004) concerning a *pool of rapidly eliminated brodifacoum* appears to be a misinterpretation of the classical two compartment model which is described in most basic texts on pharmacokinetics (e.g., O'Flaherty 1981; Gibaldi and Perrier 1982). Adopting the nomenclature of Gibaldi and Perrier (1982) for the two-compartment open model, there is a biphasic pattern in the concentration of a compound in the central compartment. The first and more rapid phase is associated with redistribution from the central to the peripheral compartment. The terminal phase is associated with excretion. The influence that the peripheral compartment has on total body burden will depend on the apparent volume of distribution (VD). As VD approaches zero, the two-compartment open model approaches a simple one-compartment model. Thus, the very short halftimes in blood (analogous to the central compartment) represent redistribution to tissue and not excretion. The longer halftimes reported in liver (analogous to the peripheral compartment) reflect retention. As discussed further below, the short halftimes in blood have little to do with body burden and are not relevant to the type of model proposed by Cadmus (2004).

Based on a review of Batten and Bratt (1990), the statement that *...the toxicokinetics of brodifacoum, particularly the rates of exchange between blood, liver, and other tissues, have not been quantified even in the rat...* is incorrect. This statement leaves the impression that the kinetics of brodifacoum are poorly understood and cannot be used for the type of analysis being considered by Cadmus (2004). The study by Batten and Bratt (1990) appears to be reasonably well done and is not difficult to interpret. Blood concentrations are problematic because brodifacoum is rapidly transported from blood to tissues, including the liver. Thus, unless very high doses are used, concentrations of brodifacoum in blood will rapidly fall below detectable concentrations. If very high doses are used, toxicity (including fundamental changes in the blood compartment due to hemorrhaging) would complicate the kinetic analysis. As illustrated in Attachment 1, these problems can be readily addressed with a physiologically based pharmacokinetic model rather than reliance on an inappropriate single compartment classical pharmacokinetic model. This has the added benefit of being able to consider prey in terms of total body burden (the relevant measure for prey) and liver concentrations as a potentially useful index for exposure in the nontarget predator species.

The assertion made by Cadmus (2004) that *many mechanistic and quantitative assumptions* would be need to model the kinetics of brodifacoum is also incorrect. As also illustrated in Attachment 1, a reasonable albeit exploratory PBPK model can be developed using only the residue data from Batten and Bratt (1990). The kinetics of brodifacoum can be described reasonably well ($r^2 = 0.92$, $p = 0.015$) by a simple flow-limited PBPK model that can be

optimized with the concentration data in liver, kidney, and carcass (which account for the bulk of the brodifacoum in the rat).

The above quoted paragraph goes on to discuss the use of the first order model and the conduct of sensitivity analyses using a range of values. The value of sensitivity analyses on a structurally incorrect model is minimal. In addition, Cadmus (2004) chose a range of halftimes from 2 days to 200 days with the implication that the "real" halftimes are somewhere in this region. In terms of the body burden of the prey species, the shorter halftimes of 2 days to several days reflect the blood data and redistribution from blood to tissue. This has nothing to do with body burden. The upper range of 200 days is presumably based on liver halftimes. As reviewed by Erickson and Urban (2004), reported liver halftimes are in the range of about 74 to 350 days. While these halftimes are not directly relevant (i.e., are not whole-body halftimes) they are more applicable than the blood halftimes. Basing most of the analyses on a 50-day half-time in a simple one compartment model is inappropriate, incorrect, and will underestimate risk.

p. 21, last ¶, last bullet to p. 22, ¶1

It is unclear why toxicity studies involving dietary exposure were not considered. Simply citing a review and a technical report from the Canadian Wildlife Service (Mineau et al. 1994, 2001) is not compelling. Cadmus (2004) note: "*LC₅₀ values can be converted to LD₅₀s if assumptions are made about food ingestion during the test, but we considered that the uncertainty introduced by those assumptions would outweigh the loss of information caused by excluding LC₅₀ data.*" This reservation may not be relevant because most guideline feeding studies in birds will report food consumption values. For those that do not, there are ample data on food consumption of quail and mallards from other guideline studies. At very least, Cadmus (2004) should have discussed the feeding studies in terms of study quality.

Nonetheless, I would agree that gavage studies will generally lead to more conservative (e.g., lower) estimates of the LD₅₀ than would transformations of dietary LC₅₀ values. In reviewing the data from Batten and Bratt (1990), however, I note a very rapid but transit spike in fecal excretion on Day 1 in rats dosed at 0.15 mg/kg bw (see Batten and Bratt 1990, Table 8, p. 33). This is worrisome in terms of the risk assessment because it could be related to a pharmacologic effect associated with gavage dosing that might not be seen in more gradual dietary exposures. Additional studies would need to be reviewed to further explore this issue. At a minimum, however, Cadmus (2004) should have presented a quantitative comparison of the gavage and dietary toxicity studies and should have more fully discussed which set of data would be most appropriate. This is particularly important for brodifacoum because the anticipated exposures will approximate dietary studies more closely than gavage studies.

p. 22, ¶1

The authors should justify or at least discuss the basis for using the logistic transformation (over the more commonly used probit transformation) in the dose-response assessment. As discussed by Finney (1972), the probit model does provide a very simple and plausible underlying assumption in the analysis of quantal response data (the log-normal distribution of individual

tolerances). I am less certain that the logistic transformation of quantal data provides a similarly simple and reasonable assumption.

p. 22, ¶4

The approach to risk characterization taken by Cadmus (2004) involves comparing single dose gavage LD_{50} doses to peak body burdens from intermittent dietary exposures over a prolonged period – e.g., 90 days in most of the simulations conducted by Cadmus (2004). The underlying assumption in this approach is that toxicity is based on peak body burden. Cadmus (2004) should have discussed this assumption more critically. For some toxicants, such as fast acting agents that are rapidly excreted and do not cause cumulative damage, peak body burden or peak target organ burden may be the most appropriate exposure index. It is not clear that this is the case for brodifacoum. As reviewed by Erickson and Urban (2004), brodifacoum causes delayed mortality. The delayed mortality could be associated with turnover times for K-dependant clotting factors (II, VII, IX, and X). While modeling this mechanistically may not be feasible, the Cadmus (2004) should have discussed and possibly considered the use of TWA body burdens (e.g., Haber's Law) rather than peak body burdens. If a PBPK model were used rather than a simple one-compartment model, TWA concentrations in the liver might well be the most relevant measure of exposure. Again, the review by Erickson and Urban (2004) provides a summary of some multiple dose studies that could be useful in assessing the whether peak concentrations or some averaging of concentrations would be the most appropriate measure of exposure.

p. 23, ¶3

The comment on density dependence is reasonable. I concur with the implicit assumption that incorporating this consideration directly into the model is probably not necessary at this time.

p. 24, ¶2

The description of the key issues for "Stage 2" is correct but the approach taken in the analysis by Cadmus (2004) is questionable on both counts as detailed in my comments above.

p. 24, description of Equation 1

Equation 1 contains two key terms, PD (proportion of the diet that consists of all rodents) and PT (proportion of rodents in the diet that are treated).

The use of PD ignores other sources (non-rodents) that may be significant for some of the nontarget species. I discuss this in other comments.

PT appears to be a composite term reflecting the proportion of target rodents that are exposed to brodifacoum (termed in my comments here as PT_1) and the fraction of the target rodents that are consumed by the nontarget predator (PT_2). In other words,

$$PT = PT_1 \times PT_2$$

In an effective baiting program, PT_1 is likely to be quite high and this is acknowledged by Cadmus (2004) near the end of their report (p. 45, ¶2). As I discuss below, information is

available that could be used to set PT_1 , reasonably objectively. PT_2 is much more problematic and difficult to set objectively. Based on *professional judgement*, Cadmus (2004) appears to think that PT_2 is low and hence they suggest that the composite PT will be very low. I am not convinced that they are correct.

By not clearly distinguishing what I am terming PT_1 and PT_2 , they give the impression that the low values of PT are based on PD (proportion of the diet that consists of rodents). In other words, they appear to suggest that because only a small proportion of target rodents are consumed by a predator (PD is small), the proportion of rodents that will be exposed to brodifacoum (PT) is also small. This, of course, makes no sense and I doubt that these authors are seriously asserting this. However, as I note below, the discussion of the relationship of PD to PT is muddled and it would be clearer if the two factors influencing PT were separately discussed.

In making this distinction, I am concerned that the Cadmus (2004) report does not sufficiently consider the proportion of nontarget rodents that may be exposed. Based on rather rapid review of the Edwards studies (Edwards and Swaine 1983; Edwards et al. 1983a,b), I think that a case could be made that exposures to nontarget rodents may be high. Thus, even though the consumption of **target rodents** may indeed be low in some instances (i.e., PT_2 is small), the nontarget predator will be exposed to brodifacoum by the consumption of **nontarget rodents**. In other words, the equation that I give above, $PT = PT_1 \times PT_2$, seems to reflect how the *professional judgement* expressed in the Cadmus (2004) report lead to a low value for PT but this approach may be overly simplistic and may have mislead their judgement in not considering exposures to nontarget rodents.

p. 26, ¶2

I do not object to the use of a lognormal distribution for body weights for wildlife species. It would have been appropriate for the authors to provide some references for this assumption (which should not be difficult to find).

p. 26, ¶4 to p. 28, ¶1

The use of only PD (proportion of rodents in the diet) as an input for dose ignores both direct consumption of the bait by nontarget species as well as the consumption of other types of animals (e.g., birds and insects) that may contain toxicologically significant amounts of brodifacoum. The importance of both direct consumption of bait and the consumption on non-rodent organisms is amply demonstrated in Erickson and Urban (2004). The sole focus on rodents will underestimate risk and this underestimate could be substantial, particularly for some species of birds – e.g., the discussion of Godfrey (1985) in Erickson and Urban (2004), p. 64.

I did not have time to review all of the selected PD values. I did look at the PD value used for the coyote in some detail. The Cadmus (2004) report appears to have used a value of 0.1985 (Table 7) as the mean of a beta distribution. The PD value appears to be consistent with cited references for the consumption of all rodents by coyote. As noted in the discussion and detailed

in Table 17, there is considerable variability among the different studies as well as differences in the ways in which data are reported. All things considered, the value of about 0.2 seems to reflect the sources cited.

I have a residual concern, however, with what may not be considered in selecting the mean of about 0.2. The field studies used by Cadmus (2004) – i.e., the U.S. EPA Wildlife Exposure Factors Handbook and the Cal/Ecotox Exposure Tables – may not be representative of the consumption of rodents by wildlife in areas with atypically high rodent populations – i.e., the type of rodent infestations that might trigger the application of brodifacoum. I am not sure how or if this could be handled quantitatively in a reasonably simple model. This would require a more detailed review of the cited studies as well as other studies that may be available.

p. 28, ¶2

In the studies where Norway rats and house mice were reported (7 observations out of approximately 160 listed in Appendix A), they comprised only 0.3% to 17.8% of the rodents in the diet.

This appears to be source of the 1% to 15% assumption for **PT**, discussed further below. As clearly indicated in this sentence, however, the range of 0.3% to 17.8% refers to the proportion of the two species of target rodents in the diet of nontarget predator species. As the model developed by Cadmus (2004) clearly states – i.e., Equation 1 on p. 24 and the discussion on pp. 26-27 – the proportion of rodents in the diet is the **PD** term and not the **PT** term.

The Cadmus (2004) report appears to be suggesting that because specific rodents comprise only a small proportion of the diet in some nontarget predator species (**PD**), only a small proportion of the rodents will be exposed to brodifacoum (**PT**). This further suggests that only target rodents will be exposed to brodifacoum.

As I have noted above, **PD** cannot be used as a basis for **PT** and Cadmus (2004) should have explicitly discussed **PT** as a composite of **PT₁** and **PT₂**, as defined above. This sort of discussion could have lead the authors of the Cadmus (2004) report and would certainly lead me to considering higher values for any composite **PT** because of the consumption on nontarget rodents by predators.

p. 28, ¶3

The proportion of Norway rats and house mice that are exposed to a brodifacoum baiting program is unknown, but is likely to be much smaller than one in ten.

The value of one in ten is unsupported. This seems to explicitly postulate an ineffective baiting program – i.e., only a small proportion of the target organisms will be exposed. I assume that brodifacoum can be and typically is used effectively – i.e., a large proportion of the target rodents are killed by exposure to brodifacoum – and thus a large proportion of the target rodent

population is exposed. This assumption is reinforced by a very casual reading of the Edwards studies (Edwards and Swaine 1983; Edwards et al. 1983, 1984a,b) which also suggest that substantial numbers of nontarget species are likely to be exposed in an effective baiting program, particularly if saturation baiting methods are used. This is discussed further below.

p. 28, ¶3

In the absence of data to support any particular value of PT , we compared results of the daily dose model using PT values ranging from 1% to 15%. The dietary composition data reviewed above suggest that even the lowest of these values is probably an overestimate.

As noted above, this statement is taking data on PD (which seems in this case to be treated as the proportion of *target* rodents in the diet of some nontarget predators) and using these data to estimate a composite PT that should include both target and nontarget rodents.

As discussed subsequently by Cadmus (2004), PT is a very sensitive parameter. To base the estimate of the range of PT on an unsupported assumption is both an error and is unnecessary. As suggested above, assumptions concerning values PT could be based on the assumption that brodifacoum is being used in an effective baiting program and that both target and nontarget rodents may be exposed.

As a preliminary illustration, Edwards et al. (1984b) provide data on a relatively successful baiting program – i.e., p. 20, Table 8, Farm A. In this example, control of rats is estimated at 88%. In other words, it seems safe to assume that at least 88% of the rats were exposed – i.e., $PT = 0.88$. As discussed by Edwards et al. (1984b), the control rate of 88% is probably an underestimate because of reinfestation. A more careful review of data associated with successful baiting programs would probably result in better estimates of PT and possible error ranges associated with PT . While somewhat speculative, it seems reasonable to assert that plausible estimates of PT (which would encompass both target and nontarget rodents) would be higher and perhaps substantially higher than 0.01 to 0.15 as used by Cadmus (2004).

Plausible estimates of PT could probably be constructed from either the available field studies or from some reasonable assumption based on a plausible definition of an effective baiting program. The range of PT values used by Cadmus (2004) seems implausible.

p. 28, ¶4

The use of DPT as an all or none variable does not bother me greatly for some receptors like the coyote. For the coyote, it seems likely that a small mammal such as a rat would be completely consumed. For other receptors, such as a red-tailed hawk, I am somewhat concerned that this approach is less realistic and could possibly underestimate risk. Some of the smaller predators such as the hawk might not consume the entire rat but could selectively begin to feed on internal organs such as the liver which would likely have higher concentrations of brodifacoum than average values in the carcass (see Attachment 1). Concern that some carcasses may be only

partially consumed is reinforced by observations of partially consumed carcasses in studies of baiting applications (Edwards and Swaine 1983; Edwards et al. 1983a,b).

p. 29, ¶2

As I have noted above, I think that the Cadmus (2004) report should have at least explored a rat feeding model that could have been compared to the monitoring data. In discussing the monitoring data of Edwards (Edwards and Swaine 1983; Edwards et al. 1983a,b), the Cadmus (2004) report states that: *A total of 144 rats found dead above ground were analyzed for brodifacoum residues.* For clarity, the Cadmus (2004) report should state that these studies did not assay all of the rats that were found. Only the first two dead rats found at each farm were sampled. It is unclear what, if any, impact this would have on the distribution fitting.

p. 32, ¶1 and ¶2

See my previous comments on the use of a single compartment model for brodifacoum and the selection of halftimes. This model is structurally unsound. I suggest that the model introduces error rather than uncertainty. As noted above, the selection of the range of halftimes from 2 days to 200 days is incorrect and seems to reflect a misunderstanding of the underlying kinetics.

p. 32, ¶4

In each of the 100 inner loop iterations, a sequence of 90 independent daily doses was simulated, ...

While I have not examined the EXCEL files in detail, I doubt that any of the simulations involved 90 daily doses unless the authors are counting days in which the animal is unexposed as a *zero dose*. Typically, the 90-day simulation period would involve far fewer than 90 exposures to brodifacoum and this is why the halftimes appeared to be relatively insensitive. As I note above, the low *PT* values used in the simulations are based on a very poorly supported assumption and probably grossly underestimate exposures to nontarget species.

The utility of the inner loop is unclear and the use of averages of maximum values will tend to dampen risk. This is addressed in greater detail in the comments by Phil Goodrum and I concur with these comments.

Also, the text does not seem consistent in the number of repetitions of the inner loop. On p. 30 and here on p. 33, 1000 repetitions are specified. On p. 32 (¶4) the number of iterations in the inner loop is specified as 100. I may be missing something here but other readers may be similarly confused. This should be clarified in the document.

p. 33, ¶3

I did not focus my review on the handling and derivations of the FIR. I did note, however, that the means of the estimated FIR's were near the upper limit of the measured FIR's. While this is also noted by Cadmus (2004) in this paragraph, it should be discussed and explained. Given how I think the FIR values were derived, I am not sure why this occurred.

p. 34, ¶5

The daily dose model was therefore very sensitive to the assumed value of PT. Based on the considerations presented in Section 3.2.2, we believe that in most cases PT is likely to be less than 0.01 (fewer than 1% of all rodents in the diet have been exposed to brodifacoum), the lowest value used in our simulations.

See my detailed comments on p. 28.

p. 35, ¶3

Here the inner loop appears to be 100 again. As for the use of means from the inner loop and the dampening of risk, see the comments from Phil Goodrum.

p. 35, ¶4

I have commented at length above on the inappropriate and unnecessary use of the first-order model.

As for the influence of halftimes on body burden (again, not the *cumulative dose*) if the first-order model is used, the arithmetic is well-characterized (e.g., Goldstein et al. 1974) and should be discussed in order to allow for a clearer interpretation of the apparent insensitivity of the halftime assumptions. Assuming first-order kinetics for a single exposure, the concentration in a system at time T after exposure (C_T), can be calculated as:

$$C_T = C_0 \times e^{-kT} \quad (\text{Eq. 1})$$

To simplify notation for considering multiple exposures, Δt is defined as the interval between exposures and p is defined as $e^{-k\Delta t}$. The peak concentration immediately after the n^{th} exposure (C_n) can be calculated as:

$$C_n = C_0 \times (1 - p^n) \div (1 - p). \quad (\text{Eq. 2})$$

As $n \rightarrow \infty$, $p \rightarrow 0$, so the peak concentration after an infinite number of exposures is:

$$C_\infty = C_0 \div (1 - p). \quad (\text{Eq. 3})$$

For multiple exposures, the concentration at time T can be calculated as:

$$C_T = C_0 \frac{(1 - p^n)}{(1 - p)} \times e^{-k(T - [(n-1)\Delta t])} \quad (\text{Eq. 4})$$

Where n is the number of exposures that have occurred up to time T and Δt is the interval between exposures.

What appears to be going on in the modeling effort by Cadmus (2004) is the Δt (the interval between doses) is typically relatively long. This decreases the value of p which in turn decreases the value of C_r . All of this occurs because of the low (and implausibly low) values of PT that were selected by Cadmus (2004).

p. 36, ¶3

I comment above on the influence of T as well as Δt on the results. A more realistic and plausible selection of PT would lead to a much greater impact of the extension of the simulation period. This follows from the kinetics discussed above.

In considering the duration of the exposure, it is also worth noting again the use of TWA concentrations may be more appropriate than peak concentrations and the TWA concentrations would be more sensitive than peak concentrations even with low values of PT .

p. 37, ¶1

The toxicity of brodifacoum is relatively well-studied in mammals and birds. The discussion presented in the Cadmus (2004) document is superficial. In terms of extrapolating among species, the analysis should have at least explored the possibility of systematic (allometric) differences among species. I have quickly scanned the reviews by Erickson and Urban (2004) and WHO (1995) and I am not under the impression that systematic differences are pronounced. Nonetheless, this issue should at least be addressed in a serious risk assessment.

As I have also noted above, the decision to exclude dietary exposure studies is not well supported. The dietary studies should have been quantitatively assessed along with the gavage studies. This analysis could have supported a more objective decision on which data sets to include or exclude. As done in the Cadmus (2004) document, the decision seems arbitrary.

p. 37, last ¶ to p. 40

I will defer to Dr. Dakins on most aspects of the dose-response assessment. As I have noted above, however, the Cadmus (2004) report should at least discuss the basis for selecting the logit transformation over the probit transformation.

p. 41 ff. **Section 5.2 Results**

Given the flaws in the methods used in the Cadmus (2004) report, the utility of the results seems minimal.

The analysis is driven by the low values of PT , defined as the proportion of the rodents exposed to brodifacoum and set at values of 0.01 to 0.15. A baiting program in which only 1% to 15% of the target species are exposed to brodifacoum would be ineffective. I would agree that an ineffective baiting program, which is what Cadmus (2004) appears to model, will not, by definition, pose much of a risk to rats and other target species and, by extension, this ineffective baiting program might not pose much of a risk to nontarget species. If the rats do not eat the brodifacoum, other species which eat the rats will not be adversely affected. If there is negligible

exposure there will be negligible risk regardless of the sophistication of the dose-response modeling. As I have noted above, however, these values for *PT* are highly questionable. These very low values of *PT* combined with the unnecessary, improperly parameterized, and inappropriate use of the simple first-order model lead to results that are not useful to the risk manager or anyone else seeking to understand the risks that might be posed by brodifacoum and how these risks might be reduced.

Notwithstanding these limitations, the Cadmus (2004) report should have at very least attempted to reconcile their analysis with the rather clear and compelling body of experience with brodifacoum that clearly indicates that risks to nontarget species are not only plausible but are well-documented. An attempt to use field data should always be part of a PRA and might have lead the authors to reexamine their model and assumptions.

p. 42, Section 6: Assumptions, Uncertainties, and Limitations

Many of the points covered in this section are important issues that should be, but were not, addressed. Simply acknowledging limitations does not improve the results.

In other cases, the Cadmus (2004) report appears to simply dismiss important issues. For example, the authors state that:

Primary exposure to non-target bait feeders could not readily be incorporated into the models we used for secondary exposure. Primary exposure was considered of less concern than secondary exposure, and more manageable through refinement of bait station design and placement. (p. 42, ¶4)

I do not understand why primary exposure could not be incorporated into the model, particularly a model as simple as the one used in the Cadmus (2004) report. The review by Erickson and Urban (2004) clearly indicates that this is an important source of exposure and this is also indicated in the studies by Edwards (Edwards and Swaine 1983; Edwards et al. 1983a,b) that the authors of the Cadmus (2004) report had clearly reviewed in some detail.

Similarly, as I have noted above, secondary exposures via the consumption of contaminated invertebrates is also clearly important but is not considered in the model in the Cadmus (2004) report. While this is acknowledged (p. 42, ¶5) in this section, the acknowledgment does not improve the risk assessment.

p. 42, ¶6

I am not sure why Cadmus (2004) did not include misuse in the risk assessment. An elaboration of the potential effects of misuse can be instructive and misuse will occur.

p. 43, ¶2

This paragraph clearly describes how Cadmus (2004) elected not to handle exposures in a way that considered the potential relationship of time on exposure. As I had noted above, I think that

the original plan, which did address the time issue, would have lead to a more realistic analysis. I have examined the studies by Edwards (Edwards and Swaine 1983; Edwards et al. 1983a,b) and these studies do not appear to provide any useful information that would assist in determining how important this limitation is.

p. 43, ¶6

The effects analysis was based on data for acute mortality, not sublethal or chronic effects (see Section 2.2.3).

This is an important consideration and deserves more than a one sentence paragraph in addressing limitations. See my comments above on p. 13, ¶3 to 5.

p. 43, Last ¶

I have not looked at the data on concentrations of brodifacoum in live-trapped rats versus carcasses. I will do so if time permits.

p. 44, ¶5

As I have noted above, the approach used to estimate *PD* is based on data that may not be representative of diets in areas with rodent infestations.

p. 45, ¶2

This is a relatively clear discussion of the factors that could effect *PT*, although the second point seems a bit muddled.

p. 45, ¶3

Following a reasonably lucid paragraph in which the plausibility of *PT* approaching unity is clearly acknowledged, the assertion that *professional judgment* leads to a *PT* of 0.01 is not credible.

p. 45, last ¶

This is a clear and correct admission of limitations associated with the use of the monitoring data on the concentrations of brodifacoum in rodent carcasses. As I have noted above, limitations in some of the monitoring studies could have been more fully discussed – i.e., selecting only the first two rats found at each site in the studies by Edwards.

p. 46, ¶1

I have commented above on the use of kinetics in the “*cumulative dose*” model. In discussion limitations, the Cadmus (2004) report notes:

Although the complexities of brodifacoum toxicokinetics were not represented in the model, we considered development of a full toxicokinetic model beyond the scope of the risk assessment and also beyond the extent of available data.

Again, these authors are suggesting that the data are either too complex and/or unavailable. It is true that developing a *full toxicokinetic model* – i.e., a model that would incorporate both pharmacokinetics and the mechanism of action – is probably not required for a useful PRA and the available data on the mechanism of action at the molecular level may be insufficient to model quantitatively. This, however, does not justify the superficial and fundamentally incorrect approach taken in the Cadmus (2004) report. The reported appears to misinterpret the available information on halftimes – i.e., the difference between redistribution and excretion – and essentially ignores 16 studies on seven species. As illustrated in Attachment 1, a very casual effort has resulted in simple PBPK model that adequately describes the kinetics of brodifacoum in the rat (the target species) and which could be readily adapted to nontarget species such as the coyote. Again, acknowledging limitations that are unnecessary or that reflect an incomplete analysis does not improve the analysis or excuse the limitations.

p. 46, ¶3

Give the other problems with this analysis, I do not have a major quarrel with limiting the simulation period to 90-days. It would have enhanced the analysis if the report had attempted to justify the period in some more rigorous way.

p. 47, ¶2, **Section 6.5 Data Gaps**

This is a relatively standard and reasonably clear discussion of data gaps with one exception:

However, data are still insufficient to parameterize even a simple two compartment (body and liver) kinetic model for the rat.

I have covered this adequately above – 16 studies covering seven species are essentially ignored. Furthermore, a “*simple two compartment (body and liver) kinetic model*” does not make any sense at all in terms of classical kinetic analysis. There is very little purpose in even trying to derive a classical multi-compartment kinetic model for any species. The advantages of PBPK models over classical kinetic models (e.g., species scaling) are clear (e.g. Bruckner et al. 2004). In terms of the sufficiency of the data, a cursory examination of one study (Batten and Bratt 1990) can be used to develop a simple PBPK model for bulk of brodifacoum in a mammal – i.e., liver, kidney, and carcass (Attachment 1).

p. 47 to 48, **Section 7 Conclusions**

The Cadmus (2004) report concludes that:

The risk of brodifacoum -induced mortality in coyote, red fox, and red-tailed hawk is low, even if these species are all assumed to be highly sensitive to brodifacoum.

Within the context of the assumptions used in the Cadmus (2004) report, this conclusion is supportable but should be reworded:

Under the conditions modeled – i.e., brodifacoum is ineffective in killing rodents because most of the rodents are not exposed to substantial quantities of brodifacoum – the risks of brodifacoum to nontarget species consuming the rodents will be low.

The Cadmus (2004) report goes on to address once again the dependence of their conclusion on *PT*. This has been adequately discussed in my comments above.

If the assumption is made that the exposure of rodents to the rodenticide is negligible to low (*PT*=0.01 to 0.15), the risks to other organisms consuming the rodents will probably be negligible to low as well. This conclusion is of little use in assessing risks posed by brodifacoum used in effective rodent control programs.

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Appendix C. Original Comments from Philip E. Goodrum, PhD

My comments focus on the conceptual modeling approach and the Monte Carlo analysis.

1. Conceptual Model (Figure 1, p. 77; Section 2.2)

The conceptual model accounts for secondary exposures to mammals and birds via the consumption of target and non-target rodents. Three reasons are given for excluding primary exposures to non-target receptors (p.12):

- a. "Risks of secondary exposure...appears to be of greater concern to regulators than risk of primary exposure..."

Comment 1:

What is the basis for this statement? A citation is needed. Ignoring an exposure pathway implies that exposures are underestimated in the risk assessment. It is difficult to understand how Agency guidance would support this assumption. In fact, the statement conflicts with the Introduction (p. 9), in which the authors cite Erickson and Urban (2002), "...EPA concluded that brodifacoum poses a greater primary and secondary hazard to birds and mammals than the other active ingredients examined."

- b. "A fundamentally different exposure model would be required...because a different approach would be needed to estimate the proportion of brodifacoum-containing food in the diet."

Comment 2:

This statement suggests that the model required to estimate exposure from bait would be too complicated to develop. This is a weak argument. A simple model is offered below. Currently, the modeling approach to address secondary exposures involves an estimate of the proportion of the diet comprised of rodents (PD) on a daily basis. This is multiplied by the food ingestion rate, which is a function of the field metabolic rate (FMR). A third variable (PT) accounts for the fraction of the animal's diet that is taken from the treated area. In short, secondary exposures are a fraction of the total daily ingestion rate and the time spent in the treated area. Equation 1 (p. 21) specifies the daily dose (DD):

$$DD = FIR \times PD \times PT \times C$$

A second source of exposure (i.e., primary exposure to bait) could be accounted for in a similar fashion. Presumably, rodenticide baiting practices are based on an understanding of feeding behavior and habitat preferences of small mammals. As noted on p. 14, factors to consider would include the application method, baiting station design, and the accessibility of the bait to non-target vertebrates. The authors indicate that data could not be obtained on these factors, so the frequency of encounters with brodifacoum-containing food were represented by the variable PT. A simplifying assumption could be that non-target animals are exposed to bait only when they are in the treated area. If PT is defined by binomial with probability (p), then a random value selected from PT will be 0 or 1. If PT = 1, then the animal may also be exposed to bait. The number of exposure events could be described by a Poisson distribution. The average rate of exposure events would be based on information from baiting practices; in the absence of data, different scenarios could be explored, similar to how the authors explored the uncertainty in DD as a function of different estimates for PT. The daily dose from bait (DDb) would be:

$$DD_b = EV \times Mb \times C_b$$

where EV is the number of exposure events (characterized by a Poisson), Mb is the average dietary mass provided by the bait, and Cb is the average concentration of brodifacoum in the bait. If PT = 0 (i.e., there is no contribution of diet from the treated area), then EV = 0. Presumably, information on baiting practices is available to define Mb and Cb. According to the authors (Section 2.2.1, p. 11), brodifacoum bait may be consumed by non-target animals that feed on pelleted or wax block baits. If pellets or wax blocks provide energy, this consumption may need to be considered part of the overall diet of the non-target animal. If the contribution to diet from bait consumption is non-trivial, then the Mb could be connected to the FIR and PD terms by the following constraint:

$$Mb \leq FIR (1 - PD)$$

That is, the contribution of bait to diet cannot exceed the balance of the FIR after accounting for the consumption of rodents. Alternatively, both primary and secondary sources can be weighted so that FIR is not exceeded:

If $(FIR \times PD + Mb) > FIR$, then the total daily dose (DDt) is constrained by FIR:

$$DD_t = (DD + DD_b) \times \frac{FIR}{FIR \times PD + M_b}$$

I have limited knowledge of baiting practices; the point of this comment is that a plausible conceptual approach for quantifying primary exposures to bait can be developed with an approach that is no more complex than the method proposed for secondary exposures.

- c. "Risk reduction for non-target bait feeders is essentially a matter of bait station design and placement."

Comment 3:

Does this imply that bait stations could be located in areas that are unavailable to non-target vertebrates? According to the authors, usage scenarios (Section 2.3.1) include applying bait outdoors (around buildings, transport vehicles, and in sewers). In rural areas, outdoor use is extensive and bait would be readily available to non-target vertebrates as a source of primary exposure.

2. PT as All or Nothing

Section 3.2.2.5 describes PT as the portion of consumed rodents that are exposed to brodifacoum. In the daily dose model, this is implemented as a binomial distribution, where a value of 0 indicates that non of the rodents consumed contain brodifacoum and a value 1 indicates that all of the rodents consumed contain brodifacoum. All or nothing is an unnecessarily extreme scenario.

The authors state (p. 25), "Because a single rat would represent a substantial fraction of the daily food intake of an individual predator, intermediate values of DPT would be unlikely; that is, the day's rodent intake would be either entirely exposed or entirely unexposed."

Comment 4:

This assumption needs to be explored more fully. The distribution of FIR needs to be presented together with the distribution of body weights for rodents.

The consequence of the "all or nothing" modeling approach for determining daily doses is that a high percentage of the days yield a dose of 0, and a small percentage yield an extremely high dose. The likelihood of exposure, or equivalently, the average percentage of days in which exposure is non-zero is determined by PT. The authors vary PT from 1% to 15%.

As an aside (**Comment 5**), varying PT from 1% to 15% is not sufficient in my opinion - when exploring what-if scenarios in the absence of robust data (i.e., representative, high quality, high quantity), a broader range should be presented to risk managers. The authors may believe that 2.5% is a high estimate for PT, but they need to demonstrate the full range (1% to 100%) for others who may have a different opinion in the absence of data.

An alternative approach is to apply the PT to individual prey meals rather than to total daily events. That is, the conceptual model can be modified to simulate prey consumption events rather than total daily events. The product, $FIR \times PD$, defines the mass of rodents consumed on 1 day. This product can then be divided by the average body weight of the rodents consumed to determine the number of individual prey items that the predator needs to consume. Then apply the PT to each meal. This gives a greater chance that at least one meal is contaminated, so there will be less days with 0 doses. Also, unlike the "all or nothing" case that is modeled right now, it is less likely that ALL of the prey items contaminated on any given day. Finally, by modeling prey meals rather than average daily events, additional sources of variability may be introduced regarding the prey availability and selection.

The difference in approaches is illustrated in Figures 1, 2, and 3 for PT values of 0.025, 0.50, and 1.0, respectively. For very low values of PT (e.g., $PT < 20\%$) [illustrated by Figure 1] and very high values of PT (e.g., $PT > 90\%$) [illustrated by Figure 2], the two approaches yield essentially the same result, as one would expect. This is because the frequency of daily consumption of contaminated prey are represented by extreme cases. However, for intermediate estimates of PT (e.g., $30\% < PT < 70\%$) [illustrated by Figure 2] the difference can be significant. Table 1 summarizes the percentiles of the daily doses for $PT = 0.50$. While the mean DD is approximately the same, less than 25% of the daily estimates are 0 for the prey method. The key variable is the bioaccumulation potential of brodifacoum. The difference in modeling approaches (i.e., All or Nothing vs. Individual Prey methods) may be important if the half-life for elimination kinetics is moderate to long (> 10 days). The greater the frequency of consecutive days of non-zero exposure, the more brodifacoum can be expected to accumulate in the body of the predator or scavenger.

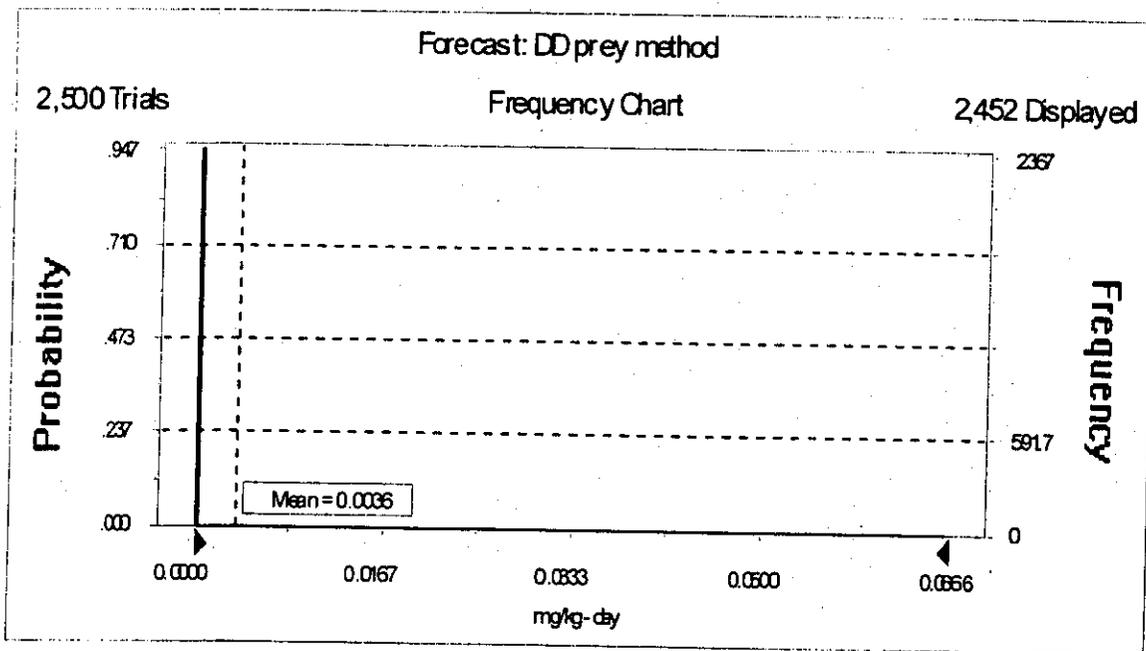
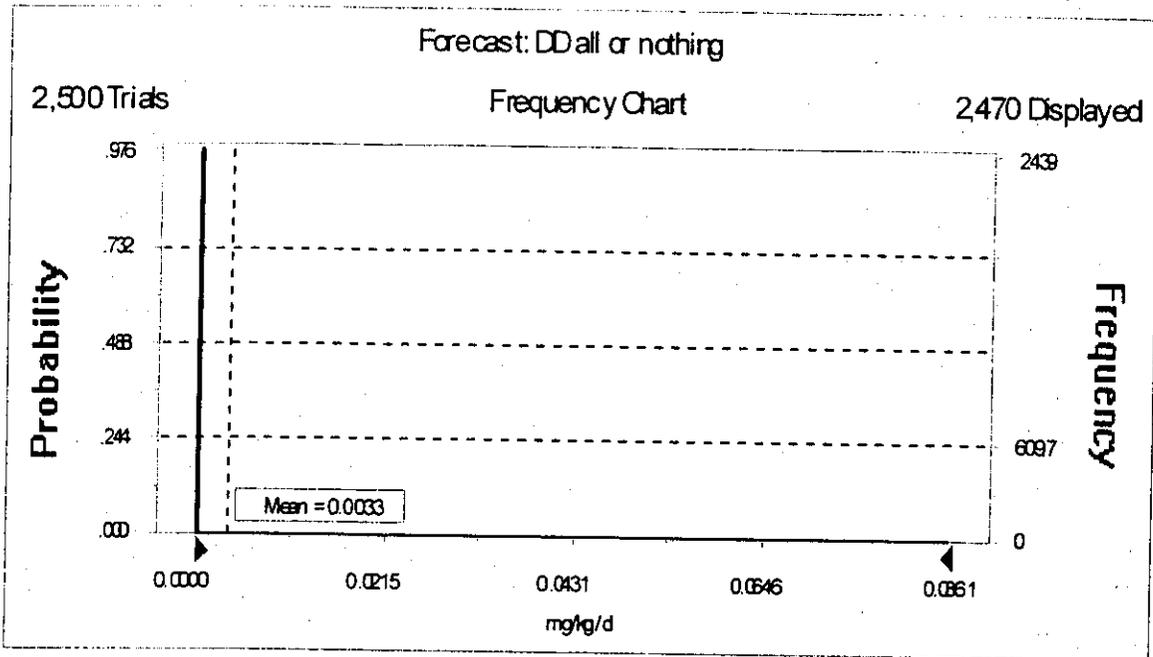


Figure 1. Comparison of approaches used to estimated daily dose to great horned owl when the proportion of rodents contaminated (PT) is set to 0.025. Average prey weight is assumed to be 50g. The "All or Nothing" approach (top panel) indicates that on any day, PT determines if all or none of the prey are contaminated. With the "prey method" (bottom panel) PT determines if any individual prey meal is contaminated; each day may result in one or more meals, some of which may be contaminated.

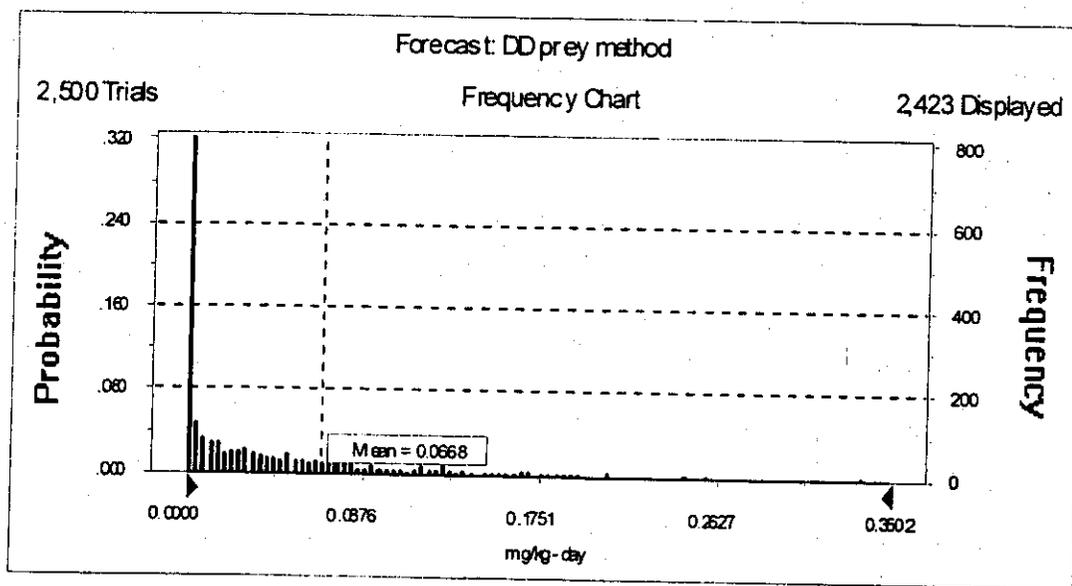
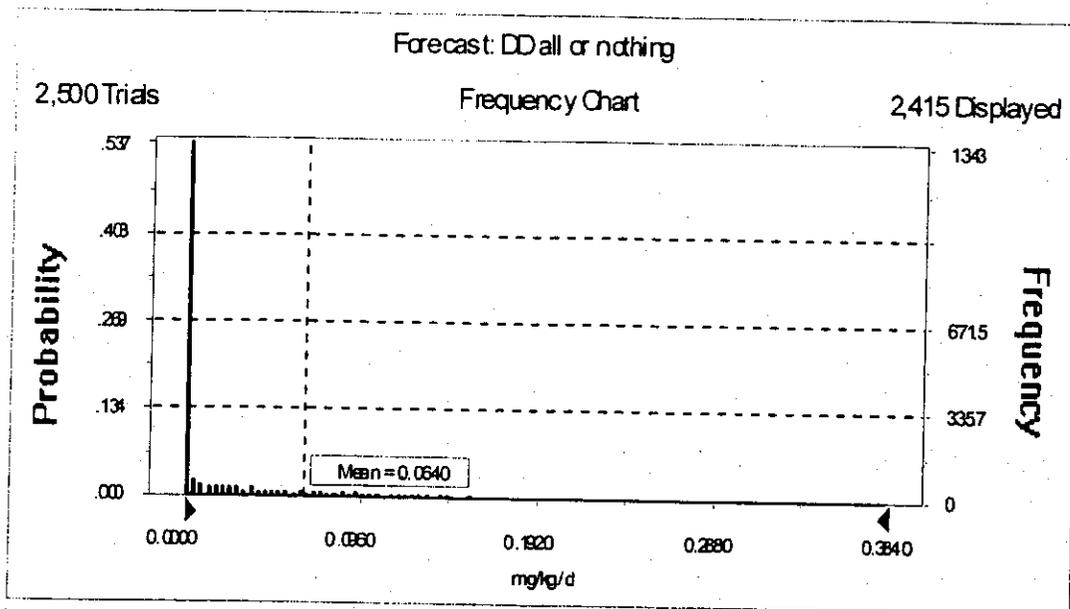


Figure 2. Comparison of approaches used to estimated daily dose to great horned owl when the proportion of rodents contaminated (PT) is set to 0.50. Average prey weight is assumed to be 50g. The "All or Nothing" approach (top panel) indicates that on any day, PT determines if all or none of the prey are contaminated. With the "prey method" (bottom panel) PT determines if any individual prey meal is contaminated; each day may result in one or more meals, some of which may be contaminated.

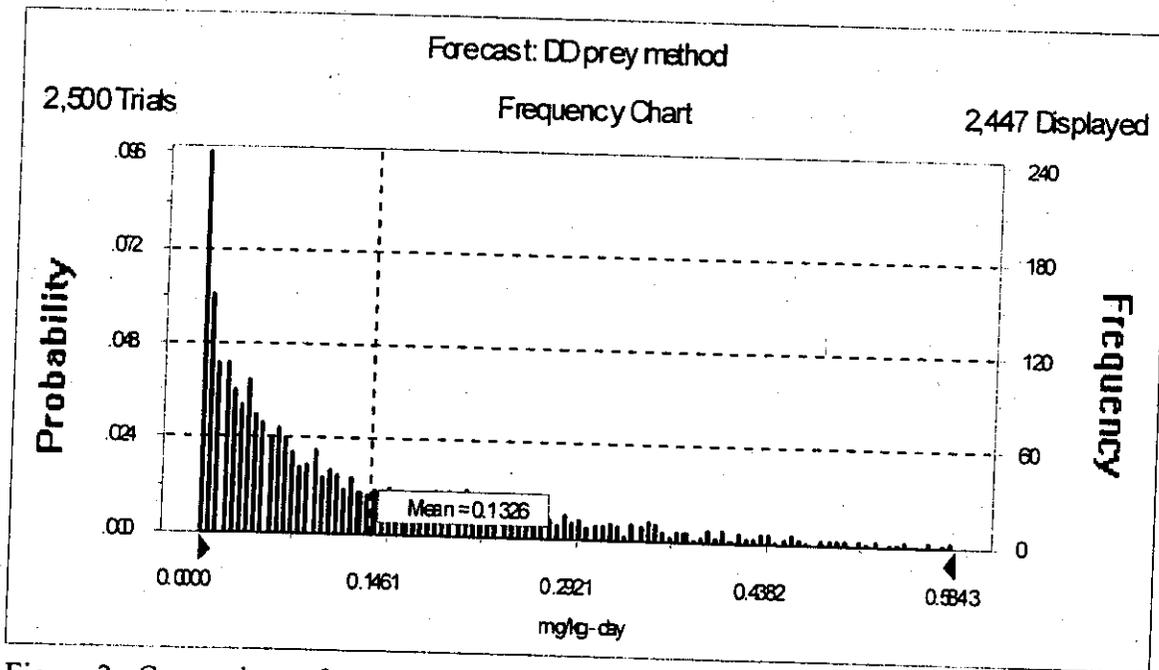
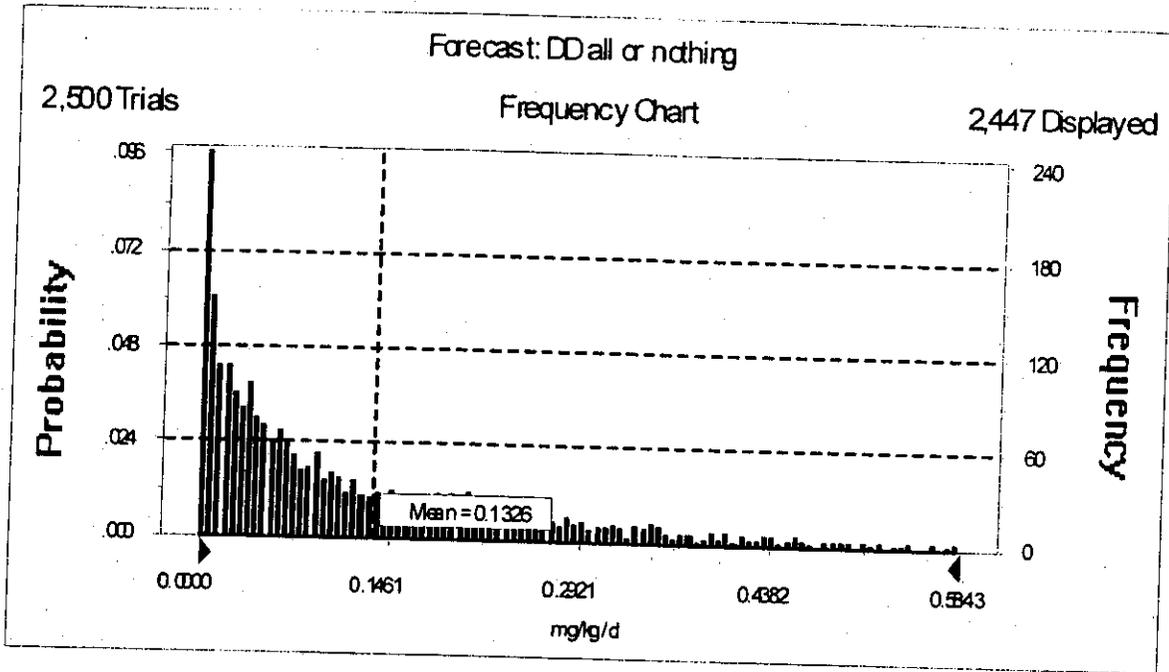


Figure 3. Comparison of approaches used to estimated daily dose to great horned owl when the proportion of rodents contaminated (PT) is set to 1.0. Average prey weight is assumed to be 50g. The "All or Nothing" approach (top panel) indicates that on any day, PT determines if all or none of the prey are contaminated. With the "prey method" (bottom panel) PT determines if any individual prey meal is contaminated; each day may result in one or more meals, some of which may be contaminated.

74

Table 1. Comparison of distributions of daily dose (mg/kg-day) for two methods of applying PT variable. All or nothing indicates prey consumed on any day are either all contaminated or none are contaminated. Prey method refers to the simulation of individual prey meals. Example is for the great horned owl, PT = 50%, 2500 iterations of the daily dose model. Corresponds to Figure 2.

Summary Statistics	All or Nothing per Day	Individual Prey Meals
Mean	0.0640	0.0668
Standard Dev	0.1294	0.1093
CV	2.0	1.6
Percentile		
0%	0.0000	0.0000
5%	0.0000	0.0000
10%	0.0000	0.0000
15%	0.0000	0.0000
20%	0.0000	0.0000
25%	0.0000	0.0004
30%	0.0000	0.0024
35%	0.0000	0.0055
40%	0.0000	0.0102
45%	0.0000	0.0162
50%	0.0000	0.0237
55%	0.0059	0.0315
60%	0.0177	0.0415
65%	0.0324	0.0522
70%	0.0495	0.0660
75%	0.0726	0.0823
80%	0.0984	0.1084
85%	0.1406	0.1395
90%	0.2135	0.1893
95%	0.3193	0.2793
100%	1.2962	1.1188

3. Monte Carlo Analysis

The use of the 2-D Monte Carlo loop to characterize inter- and intra-individual variability is an interesting and potentially informative approach. The report does not adequately describe the assumptions; without having the Excel worksheets, I would not have been able to confirm what was actually done. The power point slides provide a clearer presentation of the daily dose and cumulative dose models. The SAS code was not accompanied by the input files that were used, which also made it difficult to understand exactly how the output from the exposure assessment was used to calculate risks.

In order to evaluate the assumptions in the exposure assessment, I found it necessary to reconstruct the conceptual diagram. Figure 4 presents the entire Monte Carlo methodology (for exposure) as a series of five steps. Comments on the approach are included in the figure (see items in bullets under each step), and summarized below.

Step 1. Outer loop, Interindividual Variability.

The outer loop is used to characterize inter-individual variability in feeding behavior (percent contribution to diet of rodents) and ingestion rate.

Comment 6:

The terms $\log(a)$ and b are the intercept and slope of an allometric regression equation (Equation 3, p. 22). In the form of the regression equation, the relationship between body weight and FMR is:

$$\text{FMR} = a \text{ Wt}^b$$

$$\log(\text{FMR}) = \log(a) + b \times \log(\text{Wt})$$

or more generally

$$Y = \beta_0 + \beta_1 X$$

In this analysis, the parameters of the regression equation (β_0, β_1) are described by independent normal distributions. Typically, for simple linear regression, there is a correlation between the parameters that can be determined relatively easily. This correlation should be incorporated into the Monte Carlo simulation, as described in detail below. The consequence of ignoring the correlation depends on the direction of the correlation as well as the magnitude of the standard errors of the parameter estimates. In general, if the correlation is positive, then assuming independence will tend to yield underestimates of the response variable (Y) at the upper tail of the distribution. The opposite is true if the correlation is negative. Either way, ignoring the correlation unnecessarily constrains the variability in FMR.

Step

Specifics

1. **Outer Loop** – select parameters to define i^{th} Individual

PD ~ beta = mean for DPD
PT ~ constant = pt estimate
FIR ~ $f[\log(a); b; Wt; GE; AE; M]$ = pt estimate

log(a) ~ normal
b ~ normal
Wt ~ lognormal
GE, AE, M = pt estimates

- Characterizes inter-individual variability based on: PD, and FMR ($\log(a)$; b; Wt)
- Ignores correlation between $\log(a)$ and b – if correlation is positive, will tend to underestimate the variability in FMR

2. **Inner Loop 1** – develop time series of 1000 daily doses (DD) for i^{th} Individual. Repeat for n = 100 individuals

DPD ~ lognormal (mean from Step 1, SD)
DPT ~ binomial ($p = PT$ from Step 1) = 0,1
FIR ~ point estimate
C ~ beta

- Characterizes intra-individual variability in daily dose based on PD, PT, and C.
- PD mean is from Step 1; standard deviation (SD) is a constant. Introduces consistency for an individual (mean PD), with some day-to-day variability. By fixing SD, daily variability is assumed to be independent of mean PD. Results in extreme cases in coefficient of variation ($CV = SD/mean$); may underestimate daily variability for individuals that are high-end consumers.
- Binomial for PT defines the entire day rather than each prey meal. If DPT = 1, all prey items are contaminated, otherwise none are contaminated. With $p =$ very low probability, most days are 0 dose. For days in which prey are contaminated, this will yield very high dose (all prey are contaminated).

PD = % rodents in diet; PT = % rodents exposed to rodenticide; FIR = food ingestion rate; $\log(a)$, b = allometric scaling factors; Wt = body weight; GE = gross energy content of food; AE = assimilation efficiency; M = moisture content of food; FMR = field metabolic rate (function of $\log(a)$, b, and Wt); DPD = daily PD; DPT = daily PT, C = concentration in exposed rodents; $t_{1/2}$ = half-life for first order elimination kinetics

Figure 4. Conceptual modeling approach for the 2-D Monte Carlo analysis.

Step	Specifics
<p>3. Inner Loop 1 Summary Stats – for $n=100$ individuals, fit PDFs to mean DD and CV DD</p>	<p>Mean DD ~ beta CV DD ~ gamma</p>
<ul style="list-style-type: none"> • Defines inter-individual variability in 2-parameter lognormal distribution of DD. Mean = mean DD, SD = mean DD x CV DD. • Mean DD and CV DD are assumed to be independent for an individual; same as assumption that mean DD and SD DD are independent. 	
<p>4. Inner Loop 2 - develop time series of daily body burdens for i^{th} Individual; repeat for $n = 100$ time series; repeat for $n = 100$ individuals</p>	<p>DD ~ lognormal (mean DD, CV DD) $t_{1/2}$ ~ constant = pt estimate Body burden ~ $f(DD; t_{1/2})$</p>
<ul style="list-style-type: none"> • For i^{th} Individual, represents collective variability in DPD, DPT, and C. • For inter-individual variability, represents collective variability in mean PD and FMR. • Assumes no variability in toxicokinetics among individuals. 	
<p>5. Inner Loop 2 Summary Stats – calculate summary stats for each individual and across all individuals</p>	<ul style="list-style-type: none"> • 90-day max for each time series • 100 estimates of 90-day max for an individual > calculate mean 90-day max • 100 estimates of mean 90-day max for population > generate EDF, mean, 90th percentile
<ul style="list-style-type: none"> • 90-day max is taken from the 1st 90-days of the time series, which does not allow time for steady state. Time to steady state depends on choice of $t_{1/2}$. May underestimate the maximum daily body burden. • Mean of 90-day max is not a conservative choice. May be much lower than upper percentile or maximum of the 90-day max estimates for an individual. • EDF based on mean of 90-day max values has $N = 100$ points representing interindividual variability. How stable is the upper tail? Should N be $\gg 100$? 	

PD = % rodents in diet; PT = % rodents exposed to rodenticide; FIR = food ingestion rate; $\log(a)$, b = allometric scaling factors; Wt = body weight; GE = gross energy content of food; AE = assimilation efficiency; M = moisture content of food; FMR = field metabolic rate (function of $\log(a)$, b , and Wt); DPD = daily PD; DPT = daily PT, C = concentration in exposed rodents; $t_{1/2}$ = half-life for first order elimination kinetics

Figure 4 (cont'd). Conceptual modeling approach for the 2-D Monte Carlo analysis.

The regression statistics should be reviewed from either the Wildlife Exposure Factors Handbook, or the original study values themselves. The authors should clarify whether their intent in modeling the parameters of the regression equation (β_0, β_1) with distributions is to characterize variability or uncertainty. I believe it is better described as a source of parameter uncertainty, in which case the outer loop of the 2-D MCA describes both uncertainty and variability. This is not a critical flaw so much as a point of clarification for the risk characterization.

The steps for incorporating the correlation are presented below. Suppose that we wish to simulate a simple linear regression of the form:

$$Y = \beta_0 + \beta_1 X$$

Let b_0 and b_1 be the usual least squares estimates of β_0 and β_1 , respectively. Then, under the usual assumptions regarding the regression model, b_0 and b_1 have a joint normal distribution. The key is that b_0 and b_1 are correlated:

$$\rho = \frac{\text{Cov}(b_0, b_1)}{\sigma_{b_0} \sigma_{b_1}}$$

Without getting into the theory to support this statement, the following general approach can be used to give estimates of Y that are consistent with the idea that β_0 and β_1 are correlated parameters. First, define terms as follows:

- β_0 = intercept of the regression line ($\log(a)$ in FMR equation)
- μ_{β_0} = mean of β_0
- σ_{β_0} = standard error of β_0
- β_1 = slope of the regression line (b in FMR equation)
- μ_{β_1} = mean of β_1
- σ_{β_1} = standard error of β_1
- ρ = correlation coefficient for (β_0, β_1)

1. Generate standard normal deviate, $Z_1 \sim N(0, 1)$
2. Generate standard normal deviate, $Z_2 \sim N(0, 1)$
3. Calculate $\log(a)$

$$\log(a) = \mu_{\beta_0} + \sigma_{\beta_0} Z_1$$

4. Calculate b

$$b = \mu_{\beta_1} + \sigma_{\beta_1} [(\rho Z_1) + (1 - \rho^2)^{0.5} Z_2]$$

Assuming independence will yield the same result as setting the correlation to 0. For example, setting $\rho = 0$, yields $b = \mu_{\beta_1} + \sigma_{\beta_1} Z_2$, which is the approach currently being implemented.

For this analysis, the mean and standard errors for $\log(a)$ and b are summarized in the Cadmus (2004) report on Table 1 (p. 55). The SE for each focal species is actually quite low relative to the mean, so the consequence of ignoring the correlation is relatively minor. An example is illustrated for the FMR calculation for coyote using $\log(a) \sim N(0.412, 0.058)$, $b \sim N(0.862, 0.026)$, can $Wt \sim \text{lognormal}(9499,$

79

3600), and correlations ranging from +1 to -1. Ignoring the correlations results in differences in the tails of the distribution of FMR of about $\pm 10\%$ (see Figure 5).

Step 2. Inner Loop 1, Time Series of Daily Doses

The 2-D MCA analysis is best described as an approach that involves one outer loop and two sets of inner loops. The first inner loop is used to define a time series of daily doses for 100 individuals. The second inner loop is used to develop estimates of body burdens over time for each individual, corresponding to the variability in daily doses. In the Cadmus (2004) report, these loops are described as the Daily Dose model and Cumulative Dose model, respectively.

It is a good idea to vary daily doses for an individual, but some of the assumptions should be revisited. Comments below are on the conceptual approach to modeling daily exposures, as well as on specific assumptions regarding choices of inputs to the model.

Comment 7:

Section 3.1 (Overview of the Exposure Model) indicates that the analysis approach addresses four questions: (1) How much food does the receptor eat?; (2) What fraction of the food consists of rodents?; (3) What fraction of those rodents have been exposed to brodifacoum baits?; and (4) What is the concentration of brodifacoum in rodents exposed to baits?

The approach introduces a somewhat artificial constraint on feeding behavior. If an individual coyote is determined to have an average food ingestion rate (FIR), this implies that there is also day-to-day variability in ingestion rates. The model only accounts for daily variability in the fraction of the diet that is comprised of rodents (DPD), the concentration in the food consumed (C), and the chance of encountering contaminated prey (DPT). DPT is addressed in Comment 4 above. The authors make the point (p. 24) that diets for opportunistic predators reflect the available food supply. In simple homogeneous environments, diets are similar among individuals; in complex habitats where a wider variety of food can be found, inter-individual variability may be greater. This concept likely applies to intra-individual (day-to-day) variability as well.

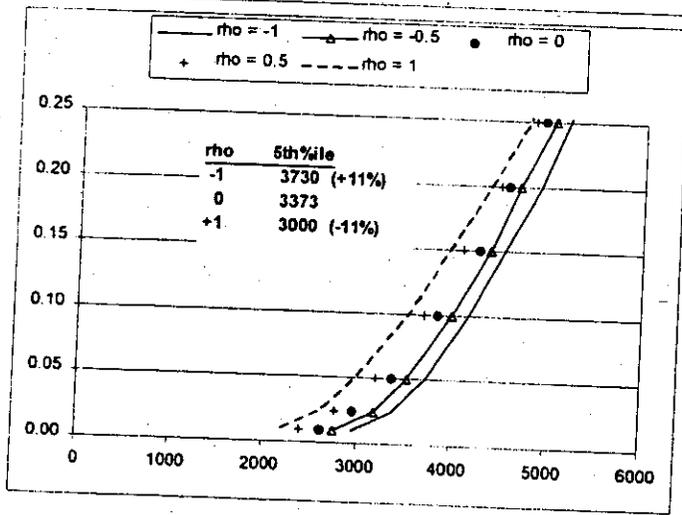
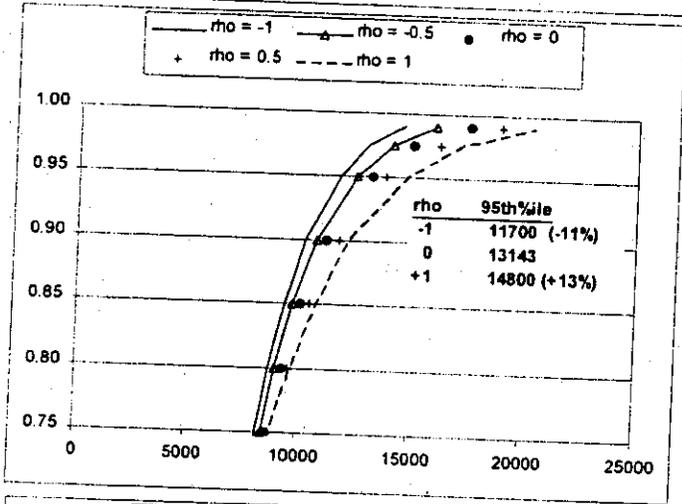
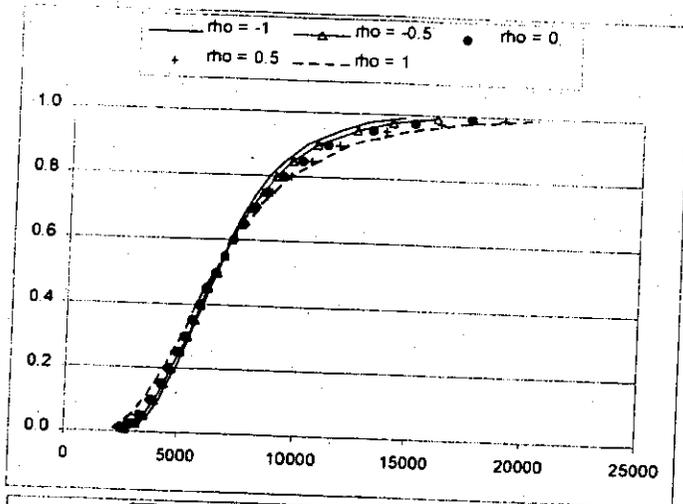


Figure 5. Distributions of FMR for coyote based on different assumptions regarding the correlation between $\log(a)$ and b in the regression equation. Top panel shows the full distribution, while the middle and bottom panels show the effect on the tails.

With a fixed estimate of FIR for all 1000 days simulated, there is no opportunity for an individual to engage in gorging. Two factors could be considered to introduce this source of variability: (1) Describe FIR with a probability distribution in which the mean is characterized by the point estimate from the outer loop, and the standard deviation is an assumption, similar to the approach used for DPD. Essentially, create a variable called DFIR; and (2) Ask an additional question of the exposure model: In order to achieve the DFIR, how many rodents (whole animals) must be consumed? It seems unrealistic to assume that a predator will eat only a fraction of its prey because it has satisfied the daily energy requirement. The concept of FIR should be interpreted as an average, with variability dependent on opportunity. The consequence of excluding this source of variability is to underestimate variability in daily dose.

Comment 8:

On p.21, the authors acknowledge the same basic model could be applied both deterministically or probabilistically. The results of the (Tier 1) deterministic (point estimate) analysis should always be presented in the same report as a more refined analysis. This information should be added to the Cadmus report if it is revised.

Comment 9:

The variability in daily dose is described as an uncertain constant by using different estimates for the standard deviation. Daily variability is assumed to follow a lognormal distribution with mean equal to the time invariant estimate of PD from the outer loop, and standard deviation equal to 3%, 5%, or 10% (pp. 24-25). This assumes that variance is independent of the mean. Regardless of whether an individual is a high or low consumer of rodents, their day-to-day variability is constant. A potential improvement would be to specify the coefficient of variation ($CV = SD/mean$) rather than the SD. In this way, the variability is in lock step with the mean. Curiously, the authors elected to use this approach in another part of the exposure model. In Step 3 (described below), distributions are fit to the time series of DD estimates. The mean DD is described by a beta and the CV DD is described by a gamma. This parameters are later multiplied to determine the corresponding SD of a lognormal distribution.

The consequence of modeling daily variability in PD with SD rather than CV is to potentially underestimate variability in individuals whose diet is comprised of a high percentage of rodents, and overestimate variability for individuals whose diet is comprised of a low percentage of rodents. Information on CV would come from studies of individual predators over time. In the absence of this information, plausible estimates of CV could be explored as a source of uncertainty in the exposure model.

Comment 10:

Also, the choice of fixed values for SD does not introduce much daily variability. It is difficult to see this without referring to the Excel sheets. The authors should include an additional table that provides not just the mean PD or the parameters of the beta distribution, but also selected percentiles and a graphic. Most readers will not be able to interpret the alpha/beta/scale parameters, but a graph would improve the transparency.

For example, Table 7 (p. 63) gives the mean PDs for each of the receptor species. The full beta distribution and selected percentiles should be provided. Referring to the Excel sheet for the Great Horned Owl (GHO_DD001.xls), cell B17 is where the beta distribution is specified. This beta is parameterized as: $\alpha = 1.04$ $\beta = 0.47$, $scale = 97.73$. The mean of this distribution is approximately

67% and the standard deviation is 28%. The interquartile range (25th, 75th percentiles) is (36%, 92%). Figure 6 provides additional information.

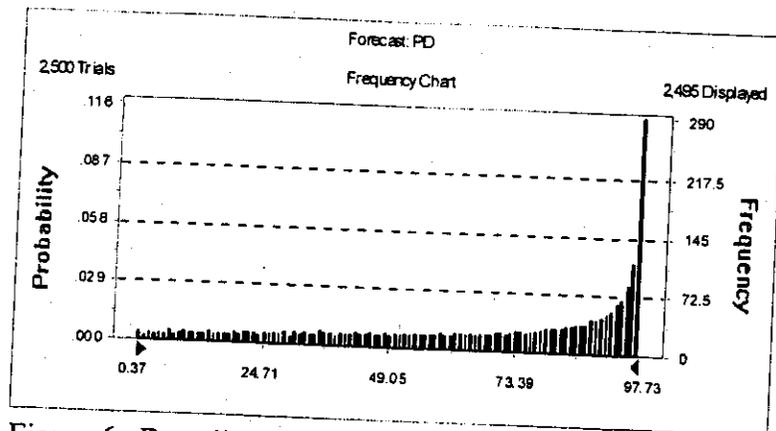


Figure 6. Beta distribution used to characterize interindividual variability in PD for great horned owl.

If this distribution is representative, this particular predator clearly exhibits great individual variability in rodent consumption. It is difficult to believe that daily variability for one individual is constrained to a standard deviation of 3% to 10%. A better approach would be to explore the idea that daily variability may be as great as 50% of the long-term average food preference. So, for a symmetric distribution, we would choose to model the mean PD \pm 50% of the mean PD. For the average horned owl with 67% of diet comprised of rodents, the standard deviation would be \pm 33%, so on any given day PD may range from 33% to 100%. This approach is equivalent to setting CV equal to 0.5.

Comment 11:

The distribution of daily doses (DPD) was characterized by a lognormal distributions. The authors state (p. 25), "The lognormal distribution was selected....because it includes only positive values and seems a reasonable choice. No data were available for direct assessment of possible distributions". The sensitivity analysis was performed only on the parameters of the SD for the lognormal. Further exploration should be done on the significance of the choice of the lognormal. Perhaps a more plausible choice in the absence of any data is a uniform distribution with (min, max) equal to \pm x%, constrained to the plausible bounds for PD (0%, 100%). Uniform distributions, in contrast to the lognormal, will select values from the high end of the range with more frequency.

Comment 12:

The authors should be sure to define any unbounded distribution like the lognormal with a maximum truncated at 100% for variables like PD that represent a percentage. I did not see this constraint included in the assumption cells of the Excel sheets. This will result in values that are greater than 100%, although given the relatively low SD for DPD, this situation is unlikely to have occurred very often.

Step 3. Inner Loop 1, Calculate Summary Statistics

For each of 100 individuals simulated with the DD model, the time series is described by a mean and CV. These parameters are fit to beta and gamma distributions, respectively (see Section 3.3.2). The time

83

series are observed to follow approximately lognormal distributions. All of these assumptions are appropriate and well described.

Comment 13:

The only possible concern is that 100 individuals is not a sufficient sample size to characterize the mean and CV. This source of uncertainty could be explored by comparing the results to a single simulation with many more individuals, or by rerunning multiple sets of 100 and comparing the summary statistics for the fitted distributions. This is not a major source of uncertainty in my opinion, given that they are focusing on mean and variance rather than upper percentiles.

Step 4. Inner Loop 2, Time Series of Cumulative Doses (Body Burdens)

The cumulative dose model describes the depuration process with a first order model. The rate constant (k), or alternatively the half-life of brodifacoum ($t_{1/2}$) is assumed to be an uncertain constant. Simulations were rerun with half-life values ranging from 5 days to 200 days to examine the effect on the exposure and risk distributions.

The model was implemented by simulating a time series of daily doses for an individual, and calculating the corresponding body burden on each day.

Step 5. Inner Loop 2, Summary Statistics

The next step is to calculate a summary statistic from the time series of body burdens. The authors suggest that 90-day maximum is an appropriate metric. For each individual, 100 sets of time series are generated, with each time series yielding an estimate of the 90-day maximum. Therefore, for 100 individuals, there are 100 sets of time series of 90-day maxima.

Comment 14:

At this point, the report gets very confusing. The reader needs to know the bottom line - what is the distribution of exposure (dose) values that is used on the calculation of the distribution of risks? There are two possible approaches to generating the distribution of doses that represents the combined sources of variability in the model:

1. **Summary Statistic Approach.** For each individual, calculate a summary statistic from the 100 sets of 90-day maximum. For example, calculate the mean 90-day maximum. Or, calculate an upper percentile (e.g., 95th %ile), or even the maximum of the maximum. The choice clearly has important implications for the overall distribution of doses and risks to the population.
2. **All 90-day Maxima Approach.** Use all of the 100 values of 90-day maxima for all 100 individuals, yielding a data set of 100 x 100 or 10,000 values to define the exposure (dose) distribution.

The Cadmus (2004) report elected Option 1, with the summary statistic being the arithmetic mean of the 90-day maxima. This yields a data set of 100 values of mean 90-day maxima.

Section 3.4.4 describes the approach, stating that "The mean of the 100 simulation outcomes was calculated, and the 100 means (from 100 individuals) were plotted as reverse cumulative frequency

distributions." But the risk characterization (Section 5.1) states, "For each cumulative dose model run, the full set of 10,000 dose estimates (100 estimates for each of 100 individuals) was combined into a single exposure distribution reflecting both within-individual and among-individual variation."

This latter statement is at best confusing, and possibly misleading. One might interpret that the earlier graphic referred to in Section 3.4.4 was for illustration only, and that when it came time to calculate risk, all 10,000 values were used as a single exposure distribution (perhaps expressed as an empirical distribution function). Indeed, Table 8 (p. 64) provides two summary statistics of the distribution of mean 90-day maxima - the mean of the means and the 90th percentile of the means. The latter statistic is described as the 10% exceedance value, which implies that the EDF that is used to calculate risk is based on the distribution of mean 90-day maxima across 100 individuals, rather than all 10,000 estimates of the 90-day maxima that were calculated. The SAS code that reads input files (which were not included in materials provided) includes loop definitions for 100 values, which further confirms the approach that was used.

Comment 15:

After establishing what was actually done, the next question is - was it a reasonable approach? There are few important observations that can be made.

The difference between the distribution of 100 values of mean 90-day maxima, and the distribution of 10,000 values of 90-day maxima are not the same. Figures 7 and 8 provide an example for coyote. For this example, setting $PT = 0.025$, half-life = 5 days, and SD of DPD = 5% (units are incorrect on the figures), the effect of decreasing variability by representing individuals by the mean 90-day max is to decrease the dose at the 5% exceedance level by 40%.

85

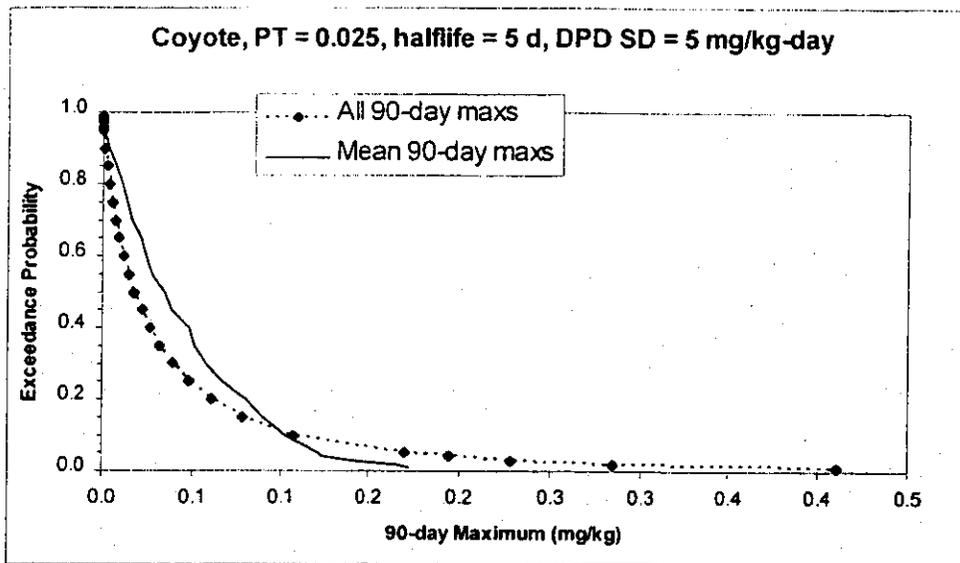


Figure 7. Comparison of two different approaches for characterizing variability in dose for coyotes. The approach used by Cadmus (2004) is to define the distribution of doses based on the mean of the 90-day maxima calculated for each of 100 individuals. At the 5% exceedance level, this yields a difference of approximately 1.4 lower than the alternative of using all estimates of 90-day maxima (see Fig 8).

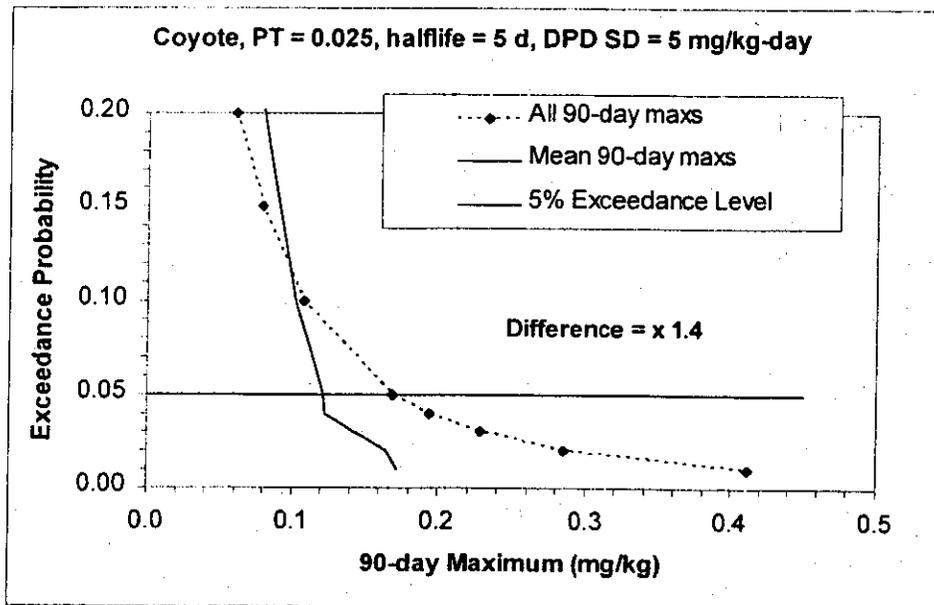


Figure 8. Same information as Figure 7, but focusing on the low end of the exceedance probability curve. The difference between approaches is greater with increasing protectiveness (lower exceedance probabilities).

Comment 16:

The choice of summary statistics is not well supported. Why choose the mean of the 90-day maxima instead of an upper percentile? What difference does this decision make? Given the importance of this choice of approaches, it should be quantified more fully in the risk assessment.

Comment 17:

The 90-day period is an arbitrary choice. Cadmus elected to select the first 90-day period for each time series of daily doses. All individuals begin the time series with a body burden of 0 mg/kg. The concept of first order kinetics implies that it may take some time for the chemical to achieve steady state. This time to steady state is influenced by both the choice of depuration half life ($t_{1/2}$) as well as the frequency of daily doses. It should be noted that for some scenarios, the frequency will be very low because PT is set to a low percentage, and because it is applied as an "all or nothing" approach (see Comment 4).

The difference between the 90-day maximum during the first 90 days and the maximum during the last 90 days of a 365 day period is demonstrated in Figures 9-11. The following observations can be made:

- When the dosing regimen is frequent, the assumption about $t_{1/2}$ becomes critical; and
- The maximum 90-day body burden is unlikely to be captured by the first 90-days of the time series, especially for longer half-life scenarios.

Comment 18:

The conclusions do not provide a comprehensive summary of the sources of uncertainty that were considered by the authors, much less the additional sources of uncertainty described above. The statement that risk of brodifacoum-induced mortality in the predator species studies is low, is unlikely to be true. Numerous examples of assumptions have been identified to illustrate that the analysis presented by Cadmus (2004) tends to underestimate exposure. Considered collectively, a reanalysis is likely to yield estimates of exposure that are greater by at least an order of magnitude.

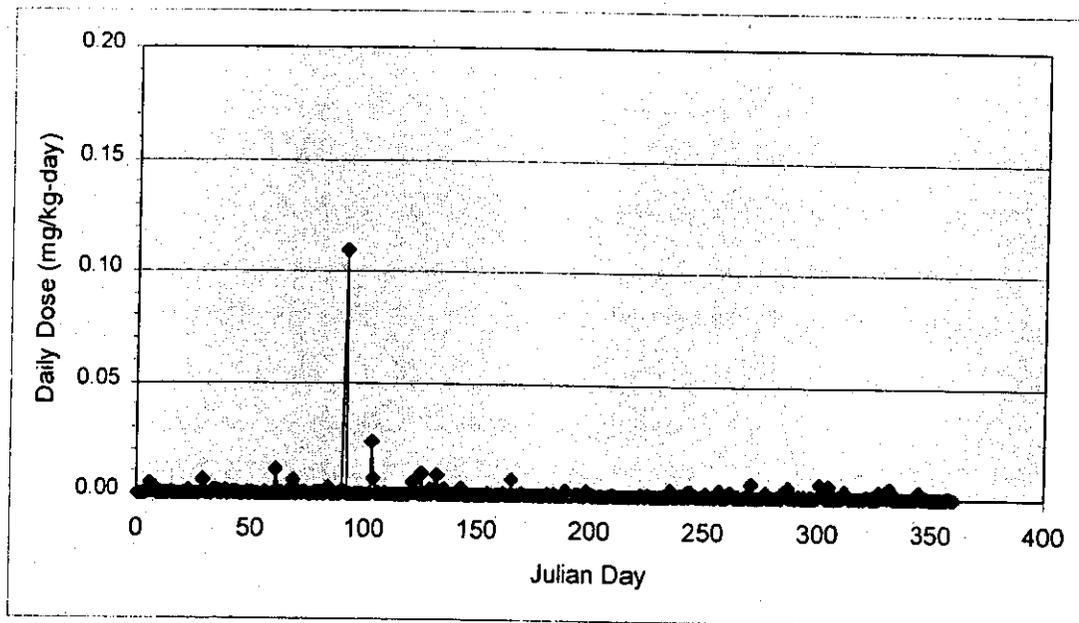


Figure 9. Example of a random 1-year time series of daily doses for a hypothetical coyote based on $PT = 0.025$ and $SD\ DPD = 5\%$.

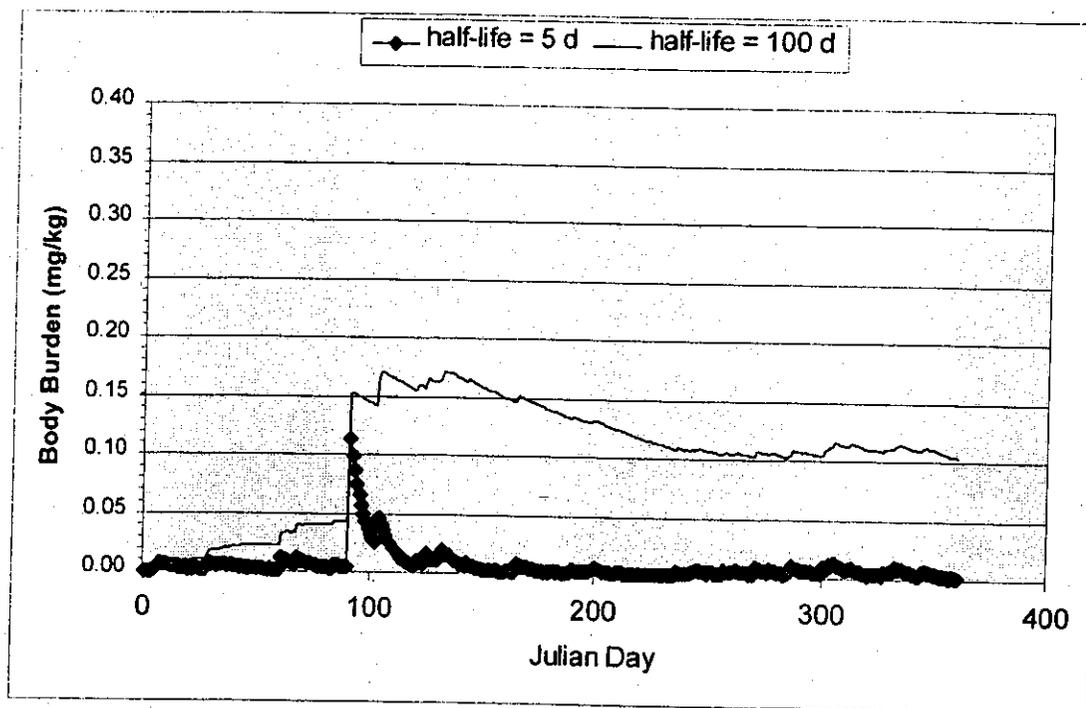


Figure 10. Comparison of the body burdens using the same dose time series (Fig 9) but two different estimates of depuration half-life. In both cases, steady state is not achieved due to the low frequency of high doses. Assuming the peak occurred during the first 90 days, it would have been captured in this example by both approaches, although the peak is higher for the scenario with slower depuration kinetics.

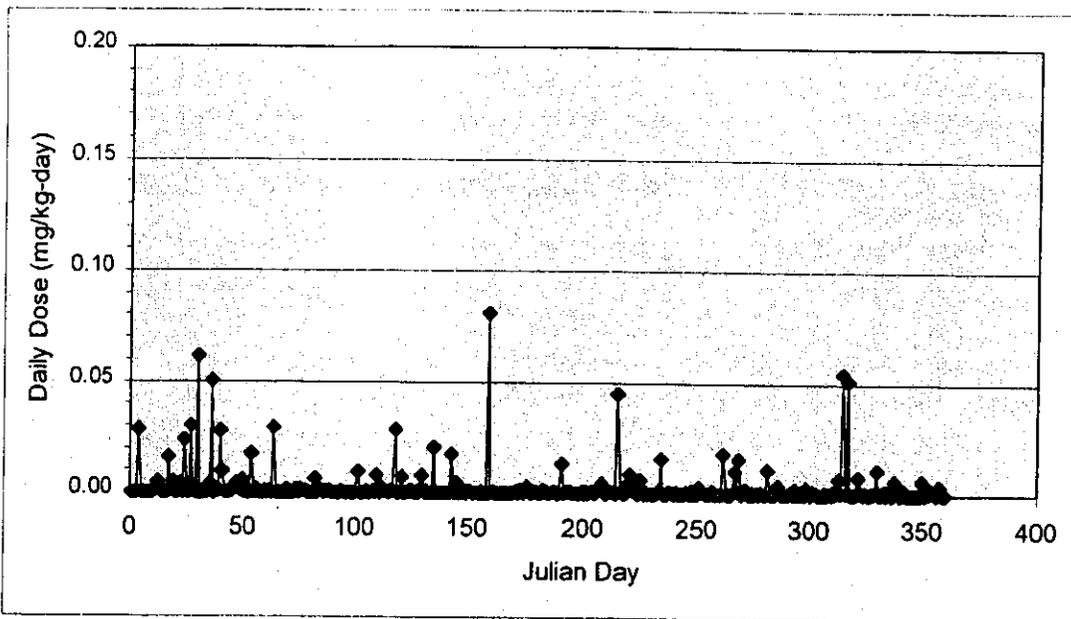


Figure 12. Example of a 1-year time series of daily doses for an individual coyote, $PT = 0.025$, $SD\ DPD = 5\%$. In contrast to the individual in Fig. 9, this individual received a lower maximum doses, but more frequent moderate doses.

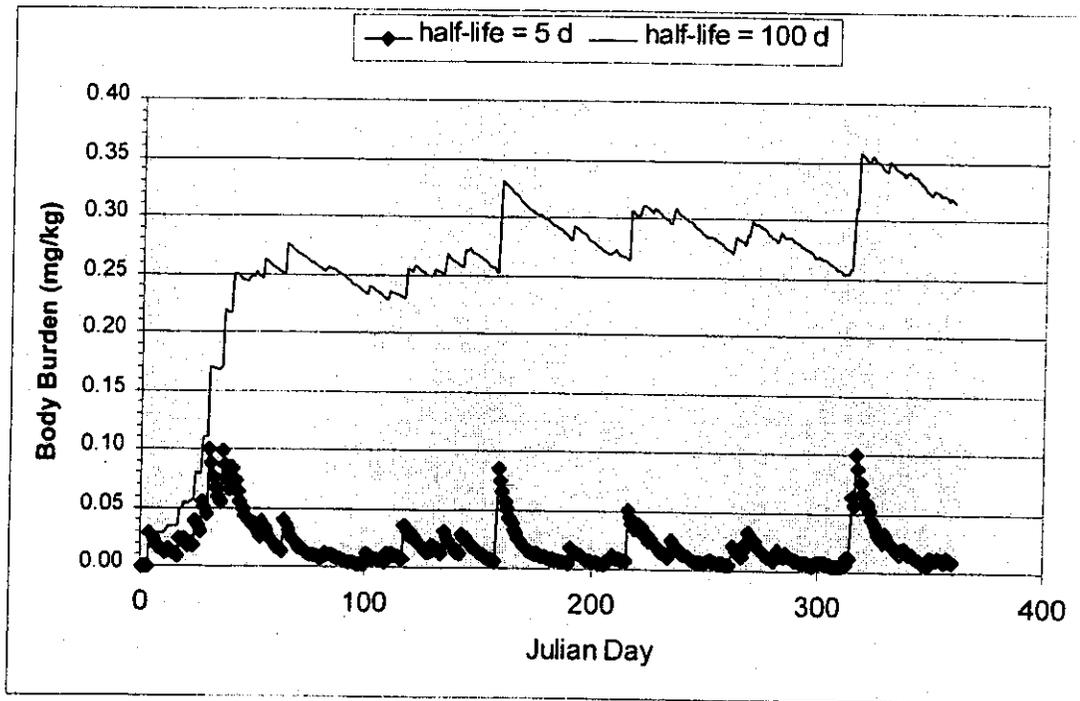


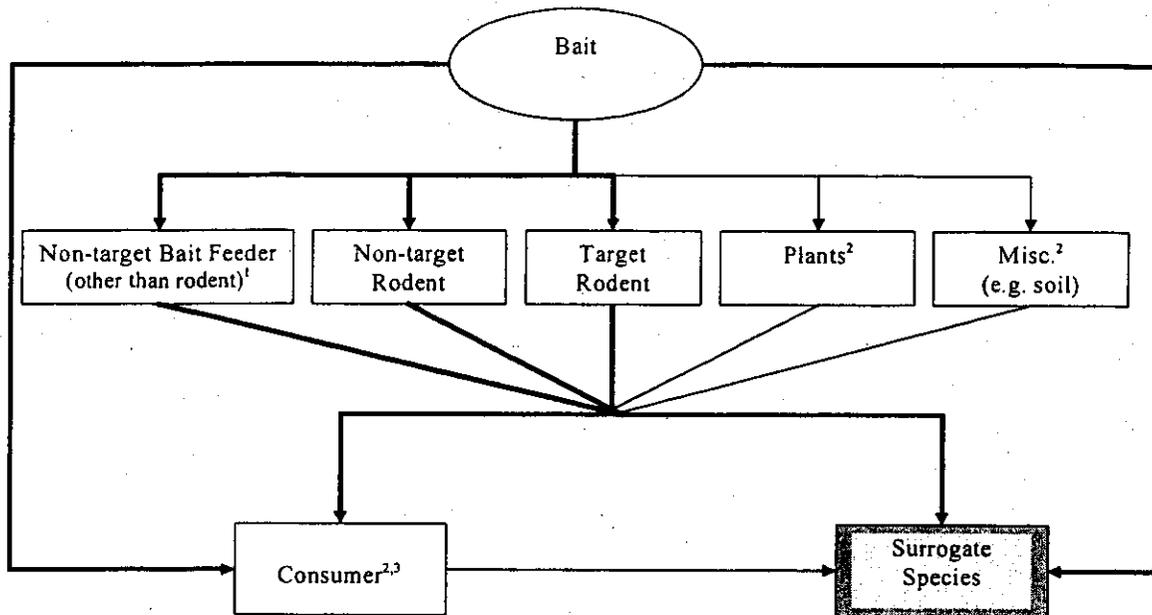
Figure 13. Comparison of daily body burdens corresponding to the time series of doses given in Fig. 12. In this case, the difference in maximum body burdens for the two scenarios is about 3.5, with the maximum body burden for $t_{1/2} = 100$ days occurring near the end of the year.

Appendix D. Original Comments from Maria Mastriano, MS

Potential Model Weaknesses

1. The conceptual model doesn't include all the potential pathways for total daily dose.
 - a. The food ingestion pathway is the only pathway considered.
 - b. Rodents are the only portion of the food ingestion pathway considered. This is not conservative. The scientific literature does not support this decision. Other food items comprise a large portion of the surrogate species' diet (e.g., rabbit, birds, invertebrates, plants, etc.)(Please see attached tables.)
 - c. No direct exposure
 - The rationale given to exclude the direct exposure pathway is not compelling. This decision in the conceptual model could cause the risk assessment to seriously underestimate risk.
2. Why were 3 mammals with very similar feeding behaviors chosen as the surrogate species? Perhaps it would be more appropriate to include predators/scavengers with more diverse eating habits.
3. The model does not cover a scenario for heavy doses of pesticide use- such as application in the open field. This was labeled a misuse of the product and was not considered in this risk assessment. Although it may be a misuse of the product (in terms of label instructions), it is plausible that this type of application occurs. Therefore, regardless of whether it is a misuse or not, it is still a potential use, and probably should be included in the risk assessment.
4. The assessment does not include population modeling. The output of the risk model is individual survival and the, "Effects on population abundance were not estimated, but must be inferred from the mortality rate." Population abundance was a major goal of the risk assessment and is a concern to EPA. Some attempt to model the effects on population should be included in the risk assessment.
5. Seasonal variability and development stage variability was not included in the model.
 - a. Data for seasonal variability is available in the open literature. Although not as abundant as composite data, seasonal data does exist. (Please see attached table 3-a study by Korschgen, 1959 which is included in EPA Wildlife Exposure Factors Handbook, 1993.) As the red fox, kit fox, and coyote are opportunistic feeders and their diets vary considerably with season, it would be better to include this seasonal variability component some where in the model.
 - b. Age (developmental stage) variability could play an important role in individual mortality and population abundance. Kits may be at a higher risk threshold both prior to weaning and post-weaning. This should be accounted for in the risk assessment.
6. Concentration-whole body vs. liver
7. Why was 90 days chosen? Which 90 days is represented? Was this an arbitrary decision? Is it missing peak risk?
8. First order depuration model

9. Assumptions for the distributions might need modification-some assumptions don't appear to be based in solid reasoning. For example, the PT was assumed to be low because of the variety and abundance of small and medium sized rodents. Variety isn't relevant here; whether there is a variety of rodents or not, it is still potential rodent prey. Secondly, abundance of rodents doesn't mean that... "the proportion of exposed rates and mice in the diet of most individuals is likely to be quite small (pg 28)." What if all the rodents are all poisoned? Also, for Norway rats, how was the 1 in 10 proportion derived?



Bold arrows represent major routes of brodifacoum to the surrogate species.

The conceptual model in the draft risk assessment does not capture all the potential pathways for total daily dose in the surrogate species. Excluding these dietary pathways is not a conservative decision and is not supported by the scientific literature. As evidenced in the attached tables, 3 of the 4 studies presented in the Wildlife Exposure Factors Handbook (EPA, 1993) illustrate that rabbits, birds, invertebrates, and plants are major dietary components (either all year or seasonally). Further, the draft figure includes detail that is irrelevant (e.g. the distinction made between a target rodent and target carcass, etc.) to the risk analysis, as explained in the draft's corresponding text to the figure. Above is a revised conceptual model. It includes all major dietary exposure pathways, and only categories that are relevant to the risk assessment.

1. Non-target bait feeders (other than rodents) which comprise a portion of the surrogate species' diet and potential total daily dose (e.g. rabbits, birds, invertebrates, etc.)
2. Plants, consumers, and misc. food items are likely to be a minor, however, perhaps not negligible source of total daily dose.
3. The consumer category is defined as non-rodent prey that consume other bait feeders (e.g., foxes consumed by other foxes and coyotes, scavenger birds (e.g. crow) by foxes, etc.)

RED FOX

TABLE 2. STOMACH CONTENTS OF 128 RED FOXES (Autumn and Winter)

Study Number	Location	Dietary Group	Percentage Occurrence	Percentage by Weight
2	Maryland Hockman JG, Chapman JA. 1983. Comparative feeding habits of red foxes (<i>Vulpes vulpes</i>) and gray foxes (<i>Urocyon cinereoargenteus</i>) in Maryland. <i>Am Midl Nat</i> 110:276-285.	Mammals	82.8	68.8
		Meadow Vole	26.6	11.3
		Eastern Cottontail	25	30.7
		White-footed Mice	12.5	1.3
		Mammal Unclassified	11.7	4.8
		Raccoon	4.7	4.9
		Gray Squirrel	3.9	2.8
		Norway Rat	3.9	2.2
		White-tailed Deer	3.9	2.5
		Domestic Cow	3.9	4.8
		Striped Skunk	3.1	1.5
		Woodland Jumping Mouse	1.6	0.2
		Muskrat	0.8	0.2
		Other	4.08	1.6
		Birds	32.8	12
		Plants	30.5	10.4
Insects	10.9	0.9		

Table 3. STOMACH CONTENTS OF 1,006 RED FOXES IN MISSOURI, 1949-1954

STUDY AREA	SEX	AGE	STOMACH CONTENTS										TOTAL				
			Rabbits	Mice and Rats	Poultry	Other mammals	Carrion	Livestock	Birds	Invertebrates	Undetermined animals	Fish and Reptiles		Plant Food	Misc.		
3	Missouri		33	24.8	24	10.7	35	36.5	41	38.7	36.8						
			46	24.2	35	6.2	58	21.3	52	22.5	22						
			25	21	35	45	13	16.3	12	11.6	13.7						
			10	4	14	1.4	11	8.1	10	8.2	7.7						
			8	12.9	7	13	6	6.5	6	7.4	7.7						
			8	9.8	3	0.3	6	2	8	5.4	5.1						
			17	0.6	10	1.2	13	1.1	15	3.8	3.4						
			12	tr.	69	15.3	13	1.6	3	tr.	0.7						
			-	-	-	-	-	2	1	0.2	0.1						
			2	tr.	1	-	1	tr.	1	0.1	tr.						
			21	2.7	35	6.9	40	6.6	20	2.1	2.7						
			-	-	-	-	2	tr.	tr.	tr.	tr.						

94

Table 4. Stomach Contents of 170 red foxes in Union County, Illinois, 1956-1967

Study Number	Location	Dietary Group	% Occurrence	% Volume
4	Illinois Knable AE. 1970. Food Habits of the Red Fox (<i>Vulpes fulva</i>) in Union County, Illinois. Transactions of the Illinois State Academy of Science 63:359-365.	Mammals	95	67.1
		Cottontail	31.8	25.2
		Prairie Vole	20.6	15.2
		Deer Mice	15.3	6.4
		House Mouse	4.7	2.5
		Fox Squirrel	2.4	2.3
		Red Fox	28.2	1
		Eastern Mole	0.6	0.9
		Pine vole	2.9	0.8
		Undetermined Mammal	8.2	0.7
		Eastern Chipmunk	1.2	0.2
		Bog Lemming	0.6	0.2
		Undetermined Rodent	2.4	T
		Other	33.7	11.6
		Birds	25.3	10.3
		Reptiles	2.4	0.3
Invertebrates	31.8	2.9		
Plants	97.1	18.6		
Misc	15.9	0.2		