Brian R. Leahy
Director

Department of Pesticide Regulation

Edmund G. Brown Jr.
Governor

M E M O R A N D U M

TO: Ann M. Prichard, Chief
Department of Pesticide Regulation
Pesticide Registration Branch

FROM: Deborah Daniels, DVM
Senior Environmental Scientist
916-322-0344

DATE: June 27, 2013

SUBJECT: SUMMARY OF SECOND GENERATION ANTICOAGULANT RODENTICIDES ASSESSMENT PEER REVIEW COMMENTS AND RESPONSES

In a July 2011 memorandum, the California Department of Fish and Wildlife (CDFW) (formerly the Department of Fish and Game) requested that the Department of Pesticide Regulation (DPR) designate all second generation anticoagulant rodenticides (SGARS) as California restricted materials. On September 19, 2012, DPR completed its final draft assessment, based on a review of available data, of the potential and actual risk to non-target wildlife from second generation rodenticides.

In accordance with Health and Safety Code section 57004, on November 21, 2012, DPR requested external peer review of its assessment entitled, "Second Generation Anticoagulant Rodenticides." DPR sent the assessment to the California Environmental Protection Agency's external peer review coordinator in the State Water Resources Control Board, Dr. Gerald Bowes. Mr. Bowes chose four peer reviewers in a manner blind to DPR. At the suggestion of Mr. Bowes, DPR's peer review request (enclosed) provided the peer reviewers with a "plain English summary" of DPR's assessment, and a list of five conclusions and assumptions (charge statements):

Charge Statement No. 1: DPR was correct to include all of the California Department of Fish and Game's data.

Charge Statement No. 2: There was no significant difference between the percent of animals with brodifacoum in the rural and urban environments, but there was a difference in the animals living in natural environment. However, there is no significant difference in the occurrence of bromadiolone and difethialone in rural, urban, and natural areas, even though the rodenticides occurred less frequently in natural areas.

Charge Statement No. 3: Brodifacoum and bromadiolone present a hazard to non-target wildlife.

Charge Statement No. 4: Difethialone and difenacoum may also present a hazard related to non-target animals

Charge Statement No. 5: Big Picture: Reviewers are not limited to addressing only the specific conclusions presented above, and are asked to contemplate the following questions:

a. In reading the memorandum, are there any additional scientific issues that are part of the conclusion that are not described above. If so, please provide further comments.
b. Taken as a whole, are the conclusions that brodifacoum and bromadiolone present and that difethialone and difenacoum may present a hazard to non-target wildlife based upon sound scientific knowledge, methods, and practices?

Below is a summary of each peer reviewer's comments and DPR's responses to those comments:

John Elliott, PhD.
Research Scientist
Science & Technology Branch
Environment Canada
Pacific Wildlife Research Centre

Peer Reviewer Comment: The assessment focuses on an analysis of incident and mortality data for wildlife in relation to exposure to SGARs over the period 1995 to 2011, along with relevant land use data and rodenticide sales and use data over the period, 2006 to 2010. In addition, there are 3 appendices to the main assessment: Appendix I summarizes wildlife incident data from areas outside of California; Appendix II summarizes wildlife incident data which involved only a single animal or for which a scientific standard was not available; Appendix III is a summary of data available for two mammal species, the fisher and the badger. The assessment begins with some basic background on the rationale for rodent control and history of chemical control methods focusing mainly on anticoagulants. Some essential toxicology including mechanisms of action is then outlined for the anti-coagulants and other classes of rodenticides.

The document then provides some regulatory context including State of California initiatives and the U.S. Environmental Protection Agency's (U.S. EPA) reevaluation dating from 2004. Of particular note, the document states that in 1999 the CDFW requested a reevaluation of the SGAR compound, brodifacoum, based on evidence of adverse effects on non-target wildlife at that time. DPR prepared an issue paper and apparently recommended as risk mitigation measures that products containing brodifacoum, bromadiolone and difethialone be restricted to indoor structural use. However, the DPR reconsidered its decision based on input from industry regarding federal laws requiring rodent control around food processing establishments. This is important in the context of more recent undertakings by the U.S. EPA to re-evaluate the rodenticide group.

The document outlines some of the decisions by the U.S. EPA in its 2008 Risk Mitigation Decision (RMD). In essence, to reduce exposure and poisoning of non-target wildlife, U.S. EPA proposed restrictions on sales, package size and usage site with the intent of reducing availability

and use of SGARs by residential users. Under the U.S. EPA decision, SGARs would no longer be sold in normal retail outlets. Sale and distribution of SGARs was restricted to outlets servicing the agricultural community, and had to be sold in units of 8 lbs. or more, and labeled for use inside or within 100 feet of agricultural buildings or other agricultural structures. Of note, the 100 foot restriction doubled the original 50 foot restriction and expanded the definition of a building. The document then describes an important feature of land use in California, the large number of one to five acre "ranchettes" or hobby farms in the state, and how people residing on such properties could continue to obtain SGARs legally at farm supply outlets by purchasing the 8 lb. + packaging, putatively for the use by licensed users.

Of further note, the document states that not all SGAR registrants complied with the U.S. EPA's risk mitigation measures. In response U.S. EPA issued a draft Notice of Intent to Cancel the registration of various products. At the time of writing the DPR memorandum, it was noted that the outcome of this process was not yet clear. However, a recent posting on the U.S. EPA website from February 5, 2013 reports that U.S. EPA is indeed moving to cancel registration of these non-compliant consumer products: (http://www.epa.gov/pesticides/mice-and-rats/cancellationprocess.html).

DPR Response: Thank you for the update. It has been integrated into the new draft.

***Peer Reviewer Comment*:** In response to Charge Statement No. 1, the peer reviewer stated that a database of exposure of 1300 animals was initially compiled by DPR. That database was then culled and the omitted results put into appendices. The report then focused on data for 492 animals produced by CDFW). However, only 350 of those samples came from datasets which reported results from all tested animals, whether they contained detectable residues of SGARs (positive) or non-detectable residues (negatives). The other 142 samples were from analyses where only the positive results were reported and the total number of animals tested was not provided. *There is value in these 'positive only' results and in the opinion of this reviewer the DPR made the correct decision to include them it its analysis of exposure of non-target wildlife to SGARs.* The 'positive-only' results do contribute to the assessment of the spatial extent of wildlife exposure. The results are summarized in Table 2. There is a difference in the percent of animals with detectable residues in the total dataset, 72.9% versus in the smaller comprehensive results sample where the incidence of exposure was 97.6%. DPR applied some simple statistical tests, Chi-squared and Fisher's exact, to compare the data sets and found no statistical differences between the two. Some further analyses and comparisons of these two datasets could have been conducted, for example: 1) mapping of the spatial distribution of the 350 'complete data' samples and the 142 'positive only' samples in order to determine visually if there were any obvious differences in the origin of the samples between the two data sets; 2) calculating a histogram or breakdown by intervals of the distribution of the data on the actual liver residue concentrations between the two data sets.

An element that cannot be determined using the larger data set, which includes the positive-only results, is the incidence of detectable residues. Those results could then be compared with published data from other jurisdictions, some of which are referred to in the report (e.g. Albert et al 2010, Murray 2011). Overall, the incidence reported for California, e.g. the presence of at least one detectable SGAR in the complete sample (492 animals) at 72.9 % (Table 2) is comparable to results reported for other jurisdictions. There is some assessment of this in Appendix I: Non-California data. Some other recent reports of incidence of SGAR residues in birds of prey include: Walker et al (2009) on barn owls from the UK (Anticoagulant rodenticides in predatory birds 2009: a Predatory Bird Monitoring Scheme (PBMS) report. Centre for Ecology & Hydrology, Lancaster, UK. 17 pp), and Christensen et al (2012) on various raptors from Denmark (Archiv Environ Contam Toxicol 63: 437-444)

DPR Response: Incidence is the probability of being diagnosed with a condition over a period of time. In humans, they will use adults. Since this requires a period of time and the animals (particularly the birds) do not occur within a lifetime of each other, we cannot obtain an incidence. However, we might be able to obtain prevalence, which is the likelihood of obtaining a disease (the number of cases of the condition within the existing population).

Peer Reviewer Comment: One element lacking in this analysis was an assessment of the analytical chemistry. There is no discussion of methods used by the laboratories employed by the CDFW, whether they were consistent, or changed over time. In particular, there is no discussion of whether more recent methods had lower detection limits which could affect the comparability of older and new data sets. This problem is discussed elsewhere, for example in Dowding et al (2012, Environ Pollut 158:161–16)

Given the importance of the residue data, I would have preferred to see some assessment of the methodology and in particular the quality assurance– quality control (QA/QC) methods employed to ensure accuracy and precision of the chemistry.

DPR Response: DPR contacted CDFW and requested this information. To date, the data have not been received.

Peer Reviewer Comment: The report provides a breakdown by species and chemical in Tables 3 and 4, which is informative. Table 6 provides a summary of data from acute lethality testing expressed as LD_{50}s, derived mainly from various U.S. EPA reports, and split between birds and mammals. The most sensitive value is provided for each compound.

The difference in apparent sensitivity between birds and mammals for some compounds is noteworthy. The database for birds is quite limited in some instances. For example, Erickson and Urban (2004) tabulate LD_{50} for 12 species for brodifacoum, while for bromadiolone data are provided for only one species, the bobwhite. This is important in the context of recent data from

Rattner et al (2011, Environ. Toxicol Chem. 30:1213–1222,) and (2012, Ecotoxicology 21:832–846) for diphacinone, where an LD_{50} value of 96.8 mg/kg body weight was recently determined for the American kestrel (Falco sparverius) and 130 mg/kg for the Eastern Screech Owl (Megascops asio), much lower values than previously available for common test species such as Northern bobwhite (Colinus virginianus) and mallards (Anas platyrhynchos). That suggests acute toxicity of SGARs, such as bromadiolone or difenacoum, could be much greater to some avian species, such as birds of prey, which are particularly at risk.

DPR Response: DPR made every effort to include the most recent and publically available data, including U.S. EPA standard studies and additional species. Erickson and Urban (2004) and the U.S. EPA (2011 and 2007) document both paraphrased multiple studies, including raptor studies, and Rattner et al (2011) was a study on American Kestrels (Falco *Sparcerius*). By utilizing these studies, we have included the most up-to-date information.

Peer Reviewer Comment: On page 13, paragraph 2 there is discussion of the findings of Riley et al (2007) on the occurrence of neoteric mange in two species of wild felid from Southern California and the possible relationship with rodenticide exposure. This follows from an earlier brief discussion of sub-lethal effects of rodenticides on page 12. Riley et al (2007) presents very interesting and important data, but as those authors note, the associations between presence of mange and exposure to anticoagulant rodenticides are correlative and not supported by an experimental work. Of particular note, in the estimation of this reviewer, is the speculation by Riley et al of the potential impact of chronic anemia on susceptibility to disease, given that the essential mode of action is to affect clotting time and thus potentially chronic sub-lethal hemorrhaging. Some authors e.g. Mosterd & Thijssen (1991, Br. J. Pharmacol. 104, 531-535) have examined the effects of repeated exposure to both SGARs and FGARs on clotting (prothrombin) time in the laboratory with rats, which demonstrates the potential for prolonged coagulophathic condition in animals repeatedly exposed to sub-lethal doses of these compounds.

DPR Response: While there is no laboratory data, there is a strong correlation, with a p-value < 0.01. However, the report language was clarified (changed from association to correlation) so that it was made clear that it was not a causative link, but a strong correlative link. The other symptoms were moved to a separate paragraph to ensure that this was the case.

Peer Reviewer Comment: On page 13, paragraph 3 the authors attempt to relate dosage from laboratory studies to liver residues. This section of the report is flawed. There is no evidence to suggest that the convergence of these numbers, as discussed here, would be anything more than coincidental. As the authors point out, the dose is based on mg per kg of whole body. These anticoagulants do accumulate in the liver (the terminology used in the report is somewhat unorthodox: "…the liver collects the rodenticide…" P. 14, L1). They appear to bind to a specific saturable binding site in the microsomal fraction of the liver, and a site which is related to vitamin K reductase activity (e.g. Mosterd & Thijssen. *ibid*.). No significant correlations have

been reported between second generation anticoagulant liver residues and oral dose (e.g. Gray *et al.* 1994. Pesticid Sci 42:179 – 184). Liver residue data cannot be relied upon as an indicator of toxicosis, and should only be used to confirm exposure, largely due to the role that individual variation has in susceptibility to AR toxicosis (e.g. Murray 2011, J Zoo Wildl Med 42: 88–97). A more appropriate way to assess the toxicity is to conduct a deterministic or probabilistic risk assessment. That would involve modeling exposure and comparing the result to lethal doses from experimental studies in order to derive risk quotients. An extensive ecological risk assessment of rodenticide impacts on non-target wildlife has been conducted by EPA and the original 2004 risk assessment is cited here as Erickson and Urban 2004. Subsequently, EPA has published further updates as it has proceeded with regulatory actions (e.g. EPA. 2011. Risks of Non-Compliant Rodenticides to Nontarget Wildlife). The outcome of that exercise provides extensive support for the contention that certain SGAR compounds, specifically brodifacoum and difethialone, are hazardous to nontarget wildlife and their use involves an unacceptable degree of risk.

Page 16, paragraph 1, in consideration of my comments above, this section is not a valid assessment of the relationship between liver residues and toxicity. Again see the comment above supported in particular by the work of Murray (2011).

DPR Response: The paragraph was reformatted to emphasize that the residues in the liver indicate exposure and that the liver sequesters brodifacoum. Currently DPR does not conduct deterministic or probabilistic risk assessment. However, DPR might consider utilizing some of the U.S. EPA's analysis in another section.

Peer Reviewer Response: On page 6, the inconsistency in how the necropsies were conducted is a concern, particularly those carried out by veterinarians "without advanced training in pathology," and non-veterinarians, given the difficulty in reliable and consistent diagnosis of poisoning by anticoagulants (Murray 2011). Nonetheless, the presentation of the data on necropsy results and the summary in Table 8 are reasonable, and the incidence is consistent with reports in the literature from a number of jurisdictions.

DPR Response: The differences in the abilities of various personnel were described, and, as noted, the results were similar to those obtained in other studies (e.g., Murray (2011).

Peer Reviewer Comment: On page 17, the descriptions of relevant natural history, dietary preferences and foraging habitats, for the various avian and mammalian predators of interest are relevant and appear to be accurate. Of the 492 animals in the large inclusive data set, location to county was available for 491 of those, which came from 35 California counties and are plotted on Figure 1 (Page 20). Tables 9 to 13, Pages 20-25 summarize the spatial data on where animals were found that contained detectable rodenticide residues in liver.

In response to Charge Statement No. 2, the peer reviewer stated that *the conclusion that there are no significant differences between the incidence of brodifacoum between rural and urban environments is sound. As is the conclusion that there is a significant difference between the urban and rural environments and the natural environment*. *Similarly, the conclusion that there are no significant differences in incidence of bromadiolone and difethialone residues among the three areas is supported by the data and the statistical analyses*. An alternative analysis of this data would have been to map the occurrence of animals with detectable concentrations of the various rodenticide compounds as on overlay onto a map of population density by county or smaller subunit.

DPR Response: We might be able to map or list the animals based on the population density at the time of the animal's mortality. While, this might allow for a more visual representation of the graphical data, it would have to be done at the time of the animal's mortality and many counties have experienced shifts in the past 15 years. For instance, the population of San Diego County has increased from ~2,498,000 in 1990 to ~2,814,000 in 2000 to ~3,177,000 in 2012. Therefore, a list might be better, but would not allow for a map. However, we do not have the data in smaller subunits.

Peer Reviewer Comment: Another alternative would be to conduct a logistic regression of the data using, for example, urban versus rural location as the bionomial variables and the actual rodenticide concentration as the continuous variable.

DPR Response: Based on the available data, logistic regression did not originally appear to be a viable option. However, we will re-explore out options utilizing population density at the time of mortality.

Peer Reviewer Comment: Further data analysis of this type could have provided further support to the Charge Statement 2. On page 26, rodenticide sales and use rates are summarized. The purpose of this section is to determine absolute and relative amounts of various rodenticides used in California, and also to attempt to compare use by various sectors, e.g. licensed applicators versus non-licensed users. The uncertainties section on page 29 discusses uncertainties in the rodenticide use data for California. Among other concerns, use of rodenticides by illegal marijuana grow operations in more remote ('natural') areas could increase exposure of non-target wildlife. This was published recently in the paper by Gabriel et al (2012) and in Appendix III. In paragraph 1, line 3 there is a typo: add 'to', should read"...tie that exposure/toxicity to any particular…"

DPR Response: Text changed per comment.

Peer Reviewer Comment: In response to Charge Statement No. 3, the peer reviewer stated that, t*hese compounds do present a hazard to non-target wildlife. My rationale for agreeing with this charge statement follows:*

1. Exposure of non-target wildlife to brodifacoum and bromadiolone, particularly avian and mammalian predators and scavengers, is widespread in California and elsewhere. Exposure has been documented by measurement of detectable residues in livers of non-target animals from many jurisdictions, including the North American sites included in the literature of the report and other reports from a number of European countries. The incidence of such exposure in carcasses of non-target wildlife varies, but is commonly in the range of > 70% for the presence of at least one SGAR compound. Brodifacoum and bromadiolone are among the most commonly detected compounds. The exposure of non-target wildlife from application of SGARs for rodent control around buildings (as opposed to field use in jurisdictions such as France) appears to be more widespread in California than has been reported for most locations, with the possible exception of the U.K. That may be due in part to a greater degree of surveillance and dissemination of findings. However, as discussed in the report, the landscape of California with the presence of many small 'ranchettes' increases spatial extent of rodenticide use and therefore exposure than would be the case in less densely populated rural farming landscapes. The documented use of rodenticide by illegal marijuana grow operations may also increase the risk of exposure of non-target wildlife even in less settled areas of the state (see especially Gabriel et al 2012).

2. Exposure to brodifacoum in particular entails a risk of acute poisoning and mortality of non-target wildlife. Brodifacoum is highly toxic to all tested species of mammals and birds; it is a very hazardous chemical. Brodifacoum has been implicated in a high proportion of non-target mortalities in California. Bromadiolone is also highly toxic to mammals and has been associated with a proportion of wildlife mortalities in California. Based on available data, bromadiolone appears to be relatively less toxic to birds, but the number of species tested is limited and does not include potentially more sensitive birds of prey,

3. Animals which are not killed outright by exposure to brodifacoum and bromadiolone may suffer from sub-lethal effects**.** The mode of action of these chemicals is to affect the Vitamin K dependent blood clotting processes. Repeat exposures in particular have identified extended effects on the clotting response of the blood, as measured for example by prothrombin times, in various test species. These studies have focused on brodifacoum in some cases but also included FGAR compounds. Data from studies which focused on or included first generation compounds (e.g. Mosterd and Thijssen 1991 *ibid*., Rattner et al 2012, cited in the report) are relevant to the more potent SGAR compounds.

Other sublethal effects have been significantly correlated with exposure to SGAR, for example the work on notoedric mange in bobcats from California (Riley et al 2007).

DPR Response: The comments are noted and appreciated.

Peer Reviewer Comment: In response to Charge Statement No. 4, the peer reviewer stated, *these compounds also present a hazard to non-target wildlife. My rationale for agreeing with this charge statement follows.* The arguments made above for brodifacoum would largely apply to difethialone. The two chemicals are structurally very similar, and acute toxicity to birds and mammals is also comparable. Difethialone is reported to be somewhat less persistent (liver half lives in rats ranging from 74 to 128 days) than brodifacoum (liver half lives in rats range from 130 to 350 days, e.g. Erickson and Urban 2004). More rapid metabolism or excretion from the primary consumer would reduce the risk to the secondary consumer. However, difethialone still persists for long periods in the liver, and given its high acute toxicity could pose a similar hazard to non-target wildlife.

Similarly, parallels can be drawn between difenacoum and bromadialone. Acute toxicity to mammals is high for both these chemicals, and they can be expected to pose a similar hazard to non-target mammals. For both of these two compounds reported avian LD50s are lower than for mammals. Again, however, only a limited number of avian species have been tested and potentially more sensitive birds of prey have not been examined. Regardless, they are both toxic to birds and have been implicated in non-target wildlife poisoning incidents.

DPR Response: The comments are noted and appreciated.

Peer Reviewer Comment: To summarize the situation, anticoagulant rodenticides were designed for and are intended to kill vertebrate pests, but there is no selectivity for target species. The second generation anti-coagulant rodenticides are effectively PBT chemicals; they are persistent, bioaccumulative and toxic. As demonstrated in this and other reports, there is widespread exposure of non-target wildlife, evidence of acute poisoning and some evidence of sublethal effects. Under modern risk assessment procedures for either pesticides or other commercial chemicals, it can be assumed that their continued commercial use would not be permitted. However, due to the lack of safe and effective alternatives and the demand for control of pest rodents, they continue to be widely employed. Given the evidence summarized in the document under review and other recent assessments, every effort must be made to strictly regulate use of these chemicals, to develop more effective risk mitigation measures where they are used, and to pressure industry to develop safer alternatives.

DPR Response: The goal of this document was to assess risk, not suggest alternatives or develop mitigation measures. However, the comments are appreciated and noted. Thank you for your time and comments.

Maureen Murray, DVM, DABVP
Clinical Assistant Professor
Department of Environmental and Population Health
Cumming School of Veterinary Medicine
Tufts University

Peer Reviewer Comment: In response to Charge Statement No. 3, the reviewer stated that ample literature supports the assertion that SGARs, particularly brodifacoum and including bromadiolone, present a hazard to non-target wildlife. The assessment prepared by DPR includes a thorough review of this literature, including publications that focus on California species as well as publications focused on other states and countries to provide further context on the scope of the problem.

The DPR assessment presents strong evidence that widespread exposure of non-target species within California occurs. Data to support exposure of California non-target wildlife are drawn from a wildlife rehabilitation center, CDFW, and published literature. These data span multiple years and document exposure in multiple species of birds and mammals, predominately through secondary exposure as most species included are predators or scavengers. Species that would likely or potentially be exposed primarily are also represented.

Of the 492 animals included in this assessment, 73% were positive for residues of at least one SGAR, with brodifacoum present in 69% and bromadiolone present in 37% of these animals. This high percentage of exposure to SGARs is in accordance with published literature on non-target wildlife exposure from Massachusetts (Murray 2011) and New York (Stone et al. 1999, Stone et al. 2003), as well as provinces in Canada (Albert et al. 2009, Thomas et al. 2011). Therefore, the magnitude of non-target exposure in California is in line with other regions where reports of testing have been published. The sales/use data cited on pp. 26-29 indicating the large amount of brodifacoum being used by non-licensed persons (e.g., homeowners) supports the likelihood that this type of use is a major pathway for brodifacoum contamination of the food chain.

While the DPR assessment does an excellent job establishing the extent of SGAR exposure in non-target wildlife, the discussion of the significance of sublethal exposures would benefit from clarification. A distinction must be made between sublethal effects that are due to impaired blood clotting (i.e., related to the mechanism of action of SGARs) versus other unspecified sublethal effects that may result in a predisposition toward the development of disease. Sublethal effects due to impaired blood clotting are coagulation abnormalities that occur in a poisoned animal that does not die from the ingested dose. On page 13 of the assessment, several studies are cited to support the statement “Second generation rodenticides are known to cause lethargy, shortness of breath, anorexia, bloody diarrhea, changes in behavior, potential heart damage, and tenderness of the joints.” The assessment should be clear that the effects noted in all but one of the cited

studies (Naz et al. 2011, which discusses direct organ damage in mice due to toxicity from brodifacoum) are due to hemorrhage that has not progressed to fatality. These effects are not the result of, nor are they contributing to a separate "disease process," as stated on page 13 and again on page 31.

DPR Response: Although many of these are related or due directly (or indirectly) to coagulopathy (such as "bloody diarrhea"), the statements were clarified to make them appear less as though they were "contributing to a separate disease process."

Peer Reviewer Comment: One study, Riley et al. 2007, which is discussed along with the studies cited above (page 13), supports the claim that sublethal residues of SGARs may contribute to the development of an infectious disease in non-target species. This study found an association between severe mange in bobcats and brodifacoum residues. However, caution must be taken not to overgeneralize these findings, as in the statement on page 13 of the assessment that "this [study] indicates that a reduced immune response to pathogens and parasites" can result from anticoagulant rodenticide exposure. As noted by the authors of the study, the findings do not indicate a cause and effect relationship. In addition, there is currently no mechanism understood through which SGARs would directly impair immune system function. To this reviewer's knowledge, only one additional study has directly investigated anticoagulant rodenticide exposure and possible predisposition to infectious disease in a non-target species. Lemus et al. 2011 has asserted a link between residues of the first generation anticoagulant chlorophacinone and increased parasite burdens in great bustards (*Otis tarda*) in Spain. However, it must be noted that the sample size is small (n=10) and no mechanism to explain this link is offered.

DPR Response: DPR stated that there was an "association to mange," indicating that there was a correlation, but not a causal link. However, the language was changed so that it was made clear that it was not a causative link, but a correlative link (changing the language from association to correlation). Additionally, DPR removed the language indicating that there could be a reduced immune response and separated the other potential consequences into another paragraph.

Peer Reviewer Comment: Discussion of the effects of sublethal SGAR residues in wildlife is frequent in the overall literature, with much of the focus on birds of prey. Within this literature there is currently no consensus that sublethal SGAR exposure increases susceptibility to non-coagulopathy-related morbidity in raptors. Two studies have found no relationship between SGAR residues and poor body condition (as a marker of health and fitness) in birds of prey (Murray 2011, Albert et al. 2009). Another study examined bone density and strength in raptors in relation to SGAR residues, given that vitamin K plays a role in bone metabolism; no adverse effects on bone density were found (Knopper et al. 2007). In addition, Murray 2011 investigated the possibility that sublethal SGAR exposure may cause liver damage in birds of prey and found no evidence of direct hepatotoxicity, although hepatotoxicity has been demonstrated in mice and rats (Kumar et al. 1993, Revathi et al. 2006). This reviewer is not aware of any studies that have

examined whether direct organ damage from SGARs may occur in non-target mammals, however.

DPR Response: Multiple studies have shown organ damage and/or a statically significant difference in the organs after exposure to SGARs. For instance, Housenger and Melendez (2011) summarized the results of an acute oral LD_{50} study on brodifacoum on Richardson's ground squirrels (*Spermophilus richardsonii*), where the animals exposed (but not the control) animals were found to have hemorrhaging (internal or external) of the "liver, gall bladder, heart, caecum, small intestines, stomach, mesenteries, thoracic and abdominal walls, and the feet." Naz et al (2011) found a significant difference in the weight of the liver, heart, and kidneys, as well as the biochemistry values, of both male and female mice that were randomly selected to controls compared to those that were fed brodifacoum. And Rahmy (1993) found myocardial necrosis in both rats and falcons that had been fed brodifacoum (the rats brodifacoum and the falcons were fed the rats minus the hearts) compared to the controls.

Peer Reviewer Comment: Given that the number of mortalities directly attributed to SGARs are usually relatively small compared to the number of exposed animals (Murray 2011, Albert et al. 2009, Stone et al. 2003), the discussion of the meaningfulness of sublethal SGAR residues in non-target wildlife is important and must be clearly defined. While only one study cited in the DPR assessment, Riley et al. 2007, supports an association between SGAR residues and disease, the sublethal, hemorrhage-related effects of SGARs are supported by literature cited on pages 12 and 13 of the assessment (Eason et al. 1996, Fisher 2009, Munday and Thompson 2003), as well as by other literature (Gray et al. 1994, Newton et al. 1990, Mendehall and Pank 1980). The literature supports the argument that SGAR residues that do not result in massive, fatal hemorrhage, still impair blood clotting in ways that would likely contribute to morbidity and mortality in non-target species - particularly in the natural environment as opposed to the laboratory setting, as noted on page 12 of the assessment (and further supported by findings in Robinson et al. 2005).

DPR Response: The language was clarified to ensure that it was clear that the studies were run in the laboratory setting. However, Riley et al. 2007 found that 3 of the 4 mountain lions had hemorrhage.

Peer Reviewer Comment: Another focus for a discussion on the importance of sublethal residues that could be further elucidated in the assessment is the ability of SGARs to accumulate in an exposed animal over time (Eason et al. 1999); therefore, each exposed animal is likely an animal at risk of eventual lethal hemorrhage if repeated exposure over time occurs (Murray 2011, Stone et al. 2003).

DPR Response: This was addressed on page 3 ("these rodenticides bioaccumulate"). However it was restated on page 12 to clarify and re-express this point.

Peer Reviewer Comment: An additional caution must be expressed about comparing liver residues to LD50 data to determine potential mortality from exposure as stated on page 13 (see also Table 7, page 15). The relationship between SGAR liver residues and signs of SGAR toxicosis has been questioned (see discussion in Murray 2011; also Albert et al. 2009, Thomas et al. 2011). However, it is appropriately stated on page 16 of the assessment that evidence of coagulopathy on necropsy is necessary for a definitive diagnosis of SGAR toxicosis.

DPR Response: The paragraph was re-written to provide further clarity.
Peer Reviewer Comment: In response to Charge Statement No. 4, the reviewer stated that, given the common properties of SGARs (high toxicity, long half-lives), it is reasonable to assume that difethialone and difenacoum, if more widely used, would present a threat to non-target animals in the same manner as brodifacoum and bromadiolone. As stated in the DPR assessment, while difethialone accounts for only 1% of anticoagulant sales, residues were found in 8% of tested animals. Although, as stated on page 8 of the DPR assessment, difenacoum has not been tested for in California, Lambert et al. 2007 documented difenacoum residues in three species of raptors. The long liver half-lives of difethialone and difenacoum are well supported by the literature cited in Table 1, page 4.

Other evidence in the literature supports the potential hazard of difethialone and difenacoum. The long liver half-lives of difethialone and difenacoum are further reviewed by Petterino et al. 2001. A laboratory study conducted on barn owls (*Tyto alba*) found that the SGARs brodifacoum, difenacoum, and flocoumafen had similar toxicities in these birds (Gray et al. 1994). Another laboratory study on barn owls documented hemorrhage in birds fed difenacoum treated rats (Mendenhall and Pank 1980). Difenacoum residues have been detected in non-target wildlife in countries where this rodenticide has been in use, including: two species of owls, pole cats (*Mustela putorius*), and European hedgehogs (*Erinaceus europaeus*) in Great Britain (**Walker et al. 2008, Newton et al. 1990, Shore et al. 1999, Dowding et al. 2010**); 3 avian species and 6 mammalian species in Spain (Ines et al. 2012); and 6 avian species in Denmark (Christiansen et al. 2012).

Further, literature on the SGAR flocoumafen, which, like difethialone, is structurally similar to brodifacoum, supports the ability of SGARs as a group to persist in liver and bioaccumulate in a manner similar to brodifacoum and bromadiolone. Flocoumafen has been shown to have a liver half-life of over 100 days both in Japanese quail (*Coturnix coturnix japonica*) (Huckle et al. 1989) and in barn owls (Newton et al. 1994).

DPR Response: Thank you for the comments.

Peer Reviewer Comment: This review does not address the first two conclusions of the DPR assessment. The conclusions drawn in points 3 and 4 of this assessment—that brodifacoum and bromadiolone present and that difethialone and difenacoum may present a hazard to non-target

wildlife—are scientifically sound. The range of species found to be positive for SGAR residues in California is indicative of the widespread contamination of the food chain by these compounds. The overall literature on non-target species exposure to SGARs is in accordance with the findings presented in the DPR assessment. It should be noted that most studies and data, including the data in this assessment, focus mainly on predators and scavengers that are likely predominantly exposed to SGARs through consumption of contaminated mammalian prey. One study that focused on an insectivore, the European hedgehog (Dowding et al. 2010) found residues of SGARs in this species, raising the possibility that terrestrial food chains may be more highly contaminated than is reflected in current literature. Moreover, literature cited in Appendix II of the DPR assessment on reptile and fetal/neonatal exposure and toxicosis indicate other less investigated areas of concern.
DPR Response: The animals we received information on were primarily carnivores and omnivores, indicating the exposures were primarily by secondary or tertiary exposure (similar to hedgehogs), which drove how the paper was written. As noted, we also received information on a reptile and fetal/neonatal exposure.

Peer Reviewer Comment: As stated previously in this review, the DPR assessment's conclusions about the link between sub-lethal SGAR residues and predisposition to disease may be overgeneralized and requires clarification considering the evidence in the current literature. However, a link between anticoagulant rodenticides and disease or other non-coagulopathy-related morbidity has been investigated in relatively few species and requires further investigation (see also Vidal et al. 2009). Moreover, this point does not negate the cause for concern about non-target species exposure to SGARs due to their potential to persist, bioaccumulate, and cause morbidity and mortality in wildlife species as a result of their primary mechanism of action, which is well supported by literature cited in the DPR assessment and in this review.

DPR Response: Addressed above, per original comments.

Peer Reviewer Comment: The DPR assessment does not directly address the potential risk of rodenticides that will remain available for general consumer use if SGARs become restricted use materials, namely first generation anticoagulants (FGARs) and the non-anticoagulant bromethalin.

DPR Response: While it was not the goal of this document to suggest alternatives or assess alternatives, the comments are noted and appreciated.

Peer Reviewer Comment: A word of caution is warranted that continued monitoring be conducted to determine if these rodenticides may have a greater than anticipated effect on non-target species.

DPR Response: DPR appreciates this concern.

Peer Reviewer Comment: Regarding FGARs, recent work has indicated that birds of prey have an increased sensitivity compared to other avian species (Watanabe et al. 2010, Rattner et al. 2011, Rattner et al. 2012). Additionally, attention should be paid to the drawbacks of using standardized acute oral toxicity tests to determine the magnitude of risk to birds of prey exposed to FGARs in natural settings as opposed to laboratory settings as described by Vyas and Rattner 2012. Regarding bromethalin, research on its ability to cause secondary poisoning is minimal but has been postulated to be possible (Mastrota and Parker 2011). The lack of specific clinical signs or gross post-mortem lesions caused by bromethalin toxicosis (Pasquale-Styles et al. 2006, Dorman 1990) will make detecting non-target mortality difficult. Additionally, continued monitoring for SGARs is warranted since the predominant pathway through which SGARs enter the food chain (licensed vs. non-licensed applicator use) has not been established.

DPR Response: The comments are noted and appreciated.

Barnett A. Rattner, Ph.D.,
Senior Scientist
Patuxent Wildlife Research Center
United States Geological Survey, Beltsville Laboratory

Peer Reviewer Comment: With regard to Charge Statement No. 1, the reviewer stated that *DPR was correct to include all of the California Department of Fish and Wildlife's data.* DPR used CDFW incident data in the analysis. Portions of the data set were derived from CDFW records that were only positive for rodenticides (negative animals were excluded). This introduces bias into the analysis, as this analysis was neither a cohort study nor a case-control study.

A table of analysis results (Chi-squared Analysis of the Data presented in Table 13) is presented in DPR's Second Generation Anticoagulant Rodenticide Assessment (henceforth referred to as the "Assessment") suggesting that there are no differences between the two data sets. However, the information in data set 2 is not clearly presented in the text (Page 8, Assessment) or in Table 2 (Page 9, Assessment), and thus the analyses and results in Attachment 2 cannot be quantitatively verified.

Using Table 2 (Page 9, Assessment), one might interpret the first data set (positives and negatives for rodenticides) to be n=492 non-target animals analyzed with 359 observations testing positive for a second generation anticoagulant rodenticide (SGAR), and the second data set (only animals testing positive for rodenticides) to be n=368 of which 359 tested positive for an SGAR. Using these reported data and the comparisons described, *p*-values were recalculated using the same interactive chi-squared utility (Preacher, 2012) and using SAS® statistical software. The results from the two statistical packages were consistent and vastly different from those presented in Attachment 2 (see below summary table and Appendix 1 of this review). Moreover, the Chi-square test is for categorical binomial data (e.g., presence, absence), not prevalence data ("n & presence of any 2nd generation rodenticide") Statistical results from Preacher, (2012) and SAS® statistical software are presented in Appendix 1 of this review.

Attachment 2, Comparison 1: n & presence of any 2nd generation rodenticide; the value reported was $p = 0.765$ The data set was presumably derived from Table 2 (Page 9, Assessment). It is not clear which data are being compared. Using the following data set, and the incorrect statistical comparison (i.e., n & presence of any 2nd generation rodenticide), the estimated *p*-value = 0.0045.

	n	SGAR Positive
Samples	492	359
Positives	368	359

A comparison using presence and absence of SGARs should have been undertaken as follows:

	n	SGAR Positive	SGAR Negative
All Samples	492	359	133
Positives	368	359	9

This comparison indicates an estimated *p*-value < 0.0001. Thus, the 2 data sets (all data versus only positive) are not equivalent, which is true.

DPR Response: The numbers that are presented in Table 2 are not designed to allow one to determine if there is a difference between the two sets of data (i.e., the data that includes CDPR's data and the data that does not); those numbers are not presented in the paper. However, those numbers are presented in the table below.

	n	SGAR Positive	SGAR Negative
All Samples	492	359	133
Positives	350	248	102

Using these numbers, there is not a statistically significant difference between the two categories (p= 0.501).

Peer Reviewer Comment: With regard to Charge Statement No. 2, the peer reviewer stated that the interpretation of some of the information in Table 13 (Modified) is correct. However, there again appears to be some serious issues with the statistical analysis. Chi-square comparisons (with Yates correction for continuity) were used. The Chi-square test is to be used for categorical binomial data (e.g., presence, absence), not for prevalence data ("n & presence of any 2nd generation rodenticide"). Furthermore, no Bonferroni correction (The Bonferroni correction is a standard statistical method to counteract the problem of multiplicity, which occurs when one) was applied for multiple comparisons using the same group. Some of the analyses undertaken (e.g., 2 by 4, 3 by 4, 2 by 3) are not Chi-square tests, but are in actuality contingency analyses. Similarly, these contingency analyses should not include "total n" in the coding. Furthermore, some individuals in the dataset are counted multiple times because they tested positive for multiple rodenticides. Statistically, this could result in Type I errors.

DPR Response: Chi-square was used because the data was converted to presence/absence data, as was indicated by the "n & presence of any 2nd generation rodenticide." Additionally, if DPR utilized Bonferroni, DPR would better control for Type I errors. However, because Type I and Type II errors are inversely prepositional, Type II errors (accepting the null hypothesis when it

was false) would automatically increase. DPR understands your perspective. However, DPR does not wish to increase Type II errors by decreasing Type I errors. After conferring with our epidemiologist, DPR elected not to use Bonferroni. DPR reanalyzed the numbers with Chi-square and came to the following:

Table. Chi-squared Analysis of the data presented in Table 13[1]

Table Size	Land types	Data Analyzed	degrees of freedom	Previous p-value	Re-analysis binomial	Re-analysis p-value
2 by 4	Urban & Rural	n & presence of brodifacoum, presence of bromadiolone, & presence of difethialone	3	0.562	0.6618	Not using
2 by 4	Urban & Natural	n & presence of brodifacoum, presence of bromadiolone, & presence of difethialone	3	0.052[2b, 5]	<0.0001	Not using
2 by 4	Rural & Natural	n & presence of brodifacoum, presence of bromadiolone, & presence of difethialone	3	0.049[2c]	<0.0001	Not using
3 by 4	Urban, Rural, & Natural	n & presence of brodifacoum, presence of bromadiolone, & presence of difethialone	6	0.038[5]	<0.0001	Not using
2 by 2	Urban & Rural	n & presence of brodifacoum	1	0.663	0.1818	0.110
2 by 2	Urban & Natural	n & presence of brodifacoum	1	0.011	<0.0001	<0.001
2 by 2	Rural & Natural	n & presence of brodifacoum	1	0.010	<0.0001	<0.001
2 by 3	Urban, Rural, & Natural	n & presence of brodifacoum	2	0.028	<0.0001	<0.001
2 by 2	Urban & Rural	n & presence of bromadiolone	1	0.912	0.8719	0.531
2 by 2	Urban & Natural	n & presence of bromadiolone	1	0.082[2b, 3]	0.0345[6]	0.001
2 by 2	Rural & Natural	n & presence of bromadiolone	1	0.107[2b]	0.0345[6]	0.016
2 by 3	Urban, Rural, & Natural	n & presence of bromadiolone	2	0.127	0.0652	0.008
2 by 2	Urban & Rural	n & presence of difethialone	1	0.285[2a]	0.1174	0.169[2a]
2 by 2	Urban & Natural	n & presence of difethialone	1	0.517[2c, 5]	0.1562	0.534[2a,2b]
2 by 2	Rural & Natural	n & presence of difethialone	1	0.200[2c, 5]	0.0542	0.156[2a,2b]
2 by 3	Urban, Rural, & Natural	n & presence of difethialone	2	0.329[2a, 5]	0.02542	0.214[2a]

Peer Reviewer Comment: Using the Preacher (2001) chi-squared utility and the presented data from the Assessment, I was able to replicate the *p*-values presented in "Table. Chi-square

Analysis of the data presented in Table 13". Yates correction was used inconsistently (e.g., 2 by 2, urban & rural, n & presence of difethialone). However, the use of "n and presence of a rodenticide" in the analysis is incorrect.

DPR Response: Yates was used (calculated) by the program when the "At least 20% of expected" (but not actual) "frequencies are less than 5" or when the value of any cell number was 0.

Additionally, using the 2 by 3 squares resulted in statically significant differences between the two methods (Preacher vs. binomial) for bromadiolone and difethialone (marked in green). Therefore, the results for the 2 by 3 boxes were not used in the final analysis to avoid any potential error.

Peer Reviewer Comment: The results of a re-analysis are presented in the Table below, coding the data as presence and absence (not prevalence) using Preacher (2001) with a Bonferroni correction. The results were also checked using SAS® statistical software. Besides being a more appropriate analysis, some of the *p*-values differ in magnitude of significance and in one instance the results differ from the reported findings. Specifically, 2x4 urban & natural presence-absence of brodifacoum, bromadiolone and difethialone: $p<0.0001$. (Statistical output available upon request) The results of a re-analysis are presented in the Table below, coding the data as presence and absence (not prevalence) using Preacher (2001) with a Bonferroni correction. The results were also checked using SAS® statistical software. Besides being a more appropriate analysis, some of the *p*-values differ in magnitude of significance and in one instance the results differ from the reported findings. Specifically, 2x4 urban & natural presence-absence of brodifacoum, bromadiolone and difethialone: $p<0.0001$. (Statistical output available upon request)

DPR Response: For consistency, DPR utilized Preacher (2001), as presented above with the green highlighting areas that would result in statistical differences (which was only in 2 areas). Based on this, DPR is comfortable using the data that we have.

Peer Reviewer Comment: As stated above, DPR did not utilized Bonferroni because if DPR better controlled for Type I errors it would have allowed for Type II errors (accepting the null hypothesis when it was false) to increase, since Type I and Type II errors are inversely prepositional. Additionally, the clarity of Table 13 would benefit by addressing the following points: a. Define and describe "Unknown Areas" and "Other Areas", and their difference.

DPR Response: The "Unknown Areas" and "Other Areas" were defined and described per their descriptions in the text and tables above this table. They were included in the text in below the table for clarification.

Peer Reviewer Comment: b. Column 1, Row 7 "Total of these animals" – column heading should be "Total number of animals categorized by land use"; discrepancy between text and table; 209 animals were classified by land use in table 13, but the text (page 19) states that precise land use categories were provided for 198 animals.

DPR Response: Per the suggestion, the number of animals has been corrected.

Peer Reviewer Comment: c. Column 1, Row 8 "Average of the evaluated data" - 492 is the total number of all animals, while the values in the other columns represent the number of "positives". Replace this cell title with "Total Animals and Number (%) of Positives".

DPR Response: Changed per suggestion.

Peer Reviewer Comment: d. Footnote 2 – The specific comparisons are ambiguous. It is stated that three anticoagulant rodenticides are being analyzed "as a group". What values are being compared? Presence and absence? Multiple comparisons are being conducted. Was a Bonferroni correction used to adjust for experiment wise error?

DPR Response: Changed per suggestion/restated for clarity.

Peer Reviewer Comment: e. Footnote 3 - For column 3 (Brodifacoum), a Chi-square analysis was conducted using presence and absence classification, and using a Bonferroni correction, the results are correct (Natural<Urban=Rural).

DPR Response: Restated for clarity. DPR did not utilized Bonferroni for the reason stated above.

Peer Reviewer Comment: For column 4 (Bromadiolone), a Chi-square analysis was conducted using presence and absence classification and Natural=Urban=Rural, which is serendipitously correct, even though a Bonferroni correction was not originally applied.

DPR Response: Restated for clarity. DPR did not utilized Bonferroni for the reason stated above.

Peer Reviewer Comment: column 5 (Difethialone), a Chi-square analysis was conducted using presence and absence classification and Natural=Urban=Rural even though a Bonferroni correction was not applied.

DPR Response: Restated for clarity. DPR did not utilized Bonferroni for the reason stated above.

Peer Reviewer Comment: Footnote 4 - The specific comparisons are not clearly identified. It is stated that three anticoagulant rodenticides are being analyzed "individually". The letter superscripts (4a, 4b, 4c) do not clearly communicate which analyses were conducted, and 4d is not identified in the footnote. What values are being compared? Multiple comparisons are being conducted. Was a Bonferroni correction used to adjust for experiment-wise error?

DPR Response: Changed per suggestion. However, DPR did not utilized Bonferroni for the reason stated above.

Peer Reviewer Comment: g. Math errors exist in the percentages presented in this Table. Specifically, row 2, column 5 - (difethialone urban) should be 6.1% not 3.4%. Row 2, column 7 (diphacinone urban) should be 3.5% not 6.1%. Row 7, column 7 (diphacinone) should be 3.4% not 3.7%. Row 8, column 5 (difethialone) should be 8.3% not 3.3%.

DPR Response: The first two had already been changed, the last one was changed per the commenter's suggestion, but the third one was unable to be identified to be changed.

Peer Reviewer Comment: h. There are inconsistencies in rounding % values in several of the cells in this Table.

DPR Response: The numbers will be double-checked as time permits.

Peer Reviewer Comment: With regard to Charge Statement No 3, the peer reviewer stated that based on the scientific literature and on the information presented in the subject Assessment, SGARs (brodifacoum, bromadiolone and difethialone) have a much longer half-life in tissues (Table 1) than first generation anticoagulant rodenticides (FGARs) (e.g., diphacinone). For this reason, it is not surprising that the SGARs brodifacoum and bromadiolone were more frequently detected in tissues of non-target wildlife than diphacinone. It is not unreasonable to conclude that if the use of SGARs was limited, the incidence of non-target wildlife mortality involving rodenticides (contributing to morbidity or the likely cause of death) would likely decrease if overall use patterns remained unchanged. However, it is also possible that increased use of FGARs could result in development of resistance in target organisms, which might be countered by greater application rates, which in turn could pose a greater hazard to non-target wildlife.

DPR Response: Thank you for the comments. While it was not the goal of this document to develop mitigation measures, the comments are noted and appreciated.

Peer Reviewer Comment: With regard to Charge Statement No. 4, the peer reviewer stated that based on the scientific literature and on the information presented in the subject Assessment, the SGARs difethialone and difenacoum both have long half-life in tissues. It is not unreasonable to predict that use of difethialone and difenacoum in place of brodifacoum and bromadiolone would

not substantially reduce non-target wildlife mortality.

DPR Response: Thank you for the comments.
Peer Reviewer Comment: In response to DPR's fifth charge statement (a), the peer reviewer noted that based on recent testing and risk assessment, FGARs may be more hazardous to non-target raptors than predicted by registration studies that used bobwhite quail (*Colinus virginianus*) and mallards (*Anas platyrhynchos*). See the following papers for scientific documentation:

Rattner, B.A., K.E. Horak, S.E. Warner, D.D. Day, C.U. Meteyer, S.F. Volker, J.D. Eisemann, and J.J. Johnston. 2011. Acute toxicity, histopathology, and coagulopathy in American kestrels (*Falco sparverius*) following administration of the rodenticide diphacinone. Environmental Toxicology and Chemistry. 30:1213-1222.

Rattner, B.A., K.E. Horak, R.L. Lazarus, K.M. Eisenreich, C.U. Meteyer, S.F. Volker, C.M. Campton, J.D. Eisemann, and J.J. Johnston. 2012. Assessment of toxicity and potential risk of the anticoagulant rodenticide diphacinone using Eastern screech-owls (*Megascops asio*). Ecotoxicology. 21:832-846.

Rattner, B.A., R.L. Lazarus, K.M. Eisenreich, K.E. Horak, S.F. Volker, C.M. Campton, J.D. Eisemann, C.U. Meteyer, and J.J. Johnston. 2013. Comparative risk assessment of the first generation anticoagulant rodenticide diphacinone to raptors. Proceedings of the 25th Vertebrate Pest Conference. 25 pp. (In Press).

DPR Response: DPR made every effort to include the most recent and publically available data, including U.S. EPA standard studies and additional species (such as raptors) including Rattner's 2011 study on American Kestrels (Falco *Sparcerius*). While DPR may not have found all of the studies that are available, DPR made a good faith effort to do so. DPR appreciates the additional references, including the reference to an upcoming study to which no data is currently available.

Peer Reviewer Comment: Some background information and citations on the criteria for case classification of rodenticides as "contributing to morbidity" or the "likely cause of death" would strengthen the document. Also, it should be acknowledged that pesticide poisonings account for a small fraction of wildlife mortality events.

DPR Response: Except when it was used as a quotation, "likely cause of death" was already used in most places and was changed where it was not to ensure clarity. Additionally, morbidity was only used in two places. In one, it states that "contributed to or could be correlated to morbidity" and in the other it stated that "Morbidity or mortality due to notoedric mange" which was changed to contributed to "Potential morbidity or mortality due to notoedric mange."

Peer Reviewer Comment: In response to DPR's fifth charge statement (b), the peer reviewer noted that some of the conclusions are based on sound knowledge, methods and practices. However, there is bias in the combined data set used in this analysis. Statistical reanalysis of some of the data are required to strengthen the interpretation of the findings.

DPR Response: Statistical reanalysis has been conducted in conjunction with an epidemiologist.
Peer Reviewer Comment: The following are minor suggestions that would improve the strength of the DPR Second Generation Anticoagulant Rodenticide Assessment document:
Page 2, paragraph 2 – revise sentence 5 to read "…exposure to rodenticides may have contributed…"

DPR Response: Text changed per suggestion.

Peer Reviewer Comment: Page 3, line 4 – revise to "…can be treated with vitamin K and recover…"

DPR Response: The chemicals can be treated with vitamin K and the animal may recover, but the chemicals will not recover (the animal will). Additionally, a wild animal is unlikely to receive this treatment so the text was not changed.

Peer Reviewer Comment: Page 8, line 4 – revise - "Chi-square" not "Chi squared"

DPR Response: Text changed per suggestion.

Peviewer Comment: Table 2, revise title to "Number (and percent) of individuals with detectable rodenticide residues among animals tested". Table 2 – revise percent exposure for birds and diphacinone to 2.6%. Check per cent values for rounding inconsistencies in table.

DPR Response: Text changed per suggestion.

Peer Reviewer Comment: Table 3, revise title to "Number of individuals (n=194) of each avian species that was positive for a first or second generation rodenticide."

DPR Response: Text changed per suggestion.

Peer Reviewer Comment: Table 4, revise title to "Number of individuals (n=298) of each mammalian species that was positive for a first or second generation rodenticide."

DPR Response: Text changed per suggestion.
Peer Reviewer Comment: Page 12, paragraph 1 – Reiterate that FGARs require consecutive days of feeding to evoke a lethal dose as described on page 3 of the document. The LD50 values for

FGARs in Table 6 are based on a single day exposure, and underestimate the actual toxicity to birds and mammals. Toxicity of FGARs increases dramatically with multiple day exposure; the LD50 is an inappropriate measure of hazard to non-target species (see Vyas, N.B. and B.A. Rattner. 2012. Critique on the use of the standardized avian acute oral toxicity test for first generation anticoagulant rodenticides. Human and Ecological Risk Assessment. 18:1069-1077).

DPR Response: Without reading the article, DPR cannot agree with all of the nuances suggested by suggestion. Therefore, while the text was changed, it was not changed to include that the LD_{50} should be higher in the wild than it is in captivity.
Peer Reviewer Comment: Page 13, paragraph 3, line 8 and page 14, line 1 - revise "collects" to binds or sequesters

DPR Response: Entire section modified per the comments of the other reviewers. Sequesters utilized as appropriate.

Peer Reviewer Comment: Table 7, chlorophacinone, total number of positive samples – revise 5.9% to 5.7%. Check the table for inconsistencies in rounding % values.

DPR Response: Text changed per suggestion and after recalculation. Other values were rechecked.

Peer Reviewer Comment: Page 20, Figure 1 – add scale bar and compass rose to figure.

DPR Response: The map was designed to indicate in which counties the animals were found, not to indicate sampling locations (i.e., an exact location) or other items that would necessitate a scale. However, a compass rose was added per suggestion. A scale bar was not added, as the map was to scale. However wording was added to indicate that the map was to scale.

Peer Reviewer Comment: Page 27, final paragraph – define "Public Health", "Regulatory Pest Control", "Structural Pest Control", and "Vertebrate Pest Control".

DPR Response: Text changed to include these definitions.

Peer Reviewer Comment: Page 29, Uncertainties – mention the difficulties and confounding factors in diagnosing and classifying a rodenticide as "contributing to morbidity" or the "likely cause of death".

DPR Response: Since some of these were already made in the necropsy section, they will not be added to the Uncertainties section. However, additional difficulties will be added to the necropsy section to increase the reader's awareness of potential problems.

Peer Reviewer Comment: Pages 32-46 – complete citations including journal volume and pages numbers should be included in the bibliography.

DPR Response: Where possible and as time allowed, the bibliography was changed to include the volume number and page number.

Peer Reviewer Comment: Appendix 1 – Statistical Results for Review Point 1.

DPR Response: Thank you for your comments and evaluations.

David Stone, Ph.D.
Assistant Professor
Department of Environmental & Molecular Toxicology
College of Agricultural Sciences
Oregon State University

Peer Reviewer Comment: DPR analyzed a large dataset (n = 492 samples) from California's Department of Fish and Game (DFG) to determine the hazard posed by second generation anticoagulant rodenticides (SGARs) to non-target wildlife. DFG's dataset included samples in which only residue-positive results were reported without recording residue-negative results. In their Memorandum, DPR provides a scientifically justifiable basis for utilizing DFG's combined data. However, additional considerations in how the data are collected and characterized are discussed in ensuing comments. As part of their assessment, DPR conducted a systematic process for excluding data that was not sufficient for their analysis. Furthermore, DPR provided rationale to address possible over-representation of residue-positive samples in their dataset.

Statistical comparison between data sets: DPR used a common statistical approach to determine if significant differences exist between the two data sets (residue-positive only data and residue positive + negative data). No statistically significant differences were observed between sample size and presence of brodifacoum, bromadiolone, difethialone or the combination of SGARs, giving DPR confidence to use DFG's entire dataset of 492 samples. In their analysis, DPR aggregated 142 residue-positive only results (with an undetermined number of residue-negative samples) and 350 samples that recorded negative + positive results. Thus, the true denominator of total samples in the aggregate dataset is unknown. The lack of a true denominator leads to some ambiguity in characterizing the percent of animals testing positive for SGARs, a prominent component of DPR's overall analysis. DPR should include more information on the residue-positive + negative data set (n= 350 animals) in their Memorandum, including descriptive statistics similar to Table 2. The larger dataset is valuable, particularly in providing additional evidence of the widespread contamination of SGARs among non-target wildlife. However, the ability for this dataset to estimate proportions of positive samples among wildlife is limited.

No discussion is included in DPR's Memorandum on whether they assessed any potential violations of the assumptions for using the Chi-square test. For this analysis, it is relevant to address the extent to which animals were obtained from non-random sampling events, and if so, whether sample collection differences may have introduced bias into the results.

DPR Response: Because this was not a study written for an epidemiological journal, DPR did not include an evaluation of why Chi-square was selected as the test of choice, what assumptions were violated, and/or what bias may have been introduced. However, DPR appreciates and understand the perspective of the Peer Reviewer.

Peer Reviewer Comment: Additional information on the statistical evaluation conducted by DPR would be helpful to justify their approach and ultimate decision to use the larger dataset.

DPR Response: Please see the response provided above for additional information on why DPR elected to use the full data set. Additionally, DPR included some text (p-values) in the white paper to justify why the larger dataset was used (p=0.501 for second generation anticoagulant rodenticides and p=0.548 for brodifacoum).

Peer Reviewer Comment: Criteria for Data Evaluation: DPR was deliberate in their selection of appropriate data for their analysis. DPR outlined a process that excluded data from an original pool of nearly 1,200 animals based on certain criteria. The reasons for data rejection included: 1) studies that provided only summary information; 2) studies in which information was provided on target animals instead of non-target wildlife; 3) studies in which the data were collected from outside California; 4) samples for which no reference toxicity standards existed; and 5) a cohort of samples in which a specific incident occurred. The process by which DPR removed some available data and ultimately retained 492 animals is a scientifically defensible approach. Furthermore, 350 of the 492 incident cases (71%) were obtained from data sets that included both residue-positive and negative samples. Additional data from California on important wildlife species were made available recently (Gabriel et al. 2012; Quinn et al. 2012) and not included in DPR's analysis due to timing issues.

Underreporting of wildlife incidents: DPR addresses the issue of over representation of samples in their dataset (n= 492 animals) and concludes that these data do not over-represent residue-positive results among non-target wildlife. This statement is supported by the multiple challenges in conducting surveillance of wildlife and the likelihood for under-reporting incidents (Vyas, 1999). There are many factors that complicate monitoring for secondary poisoning as a result of exposure to SGARs (Riley et al. 2007), including the expense and sophistication required for laboratory detection of residue, as well as scavenger removal. In studies with active monitoring, scavenging played a significant role in the removal of raptor carcasses (Smallwood and Thelander, 2008; Rivera-Milan et al. 2004). Other factors such as decomposition rates, weather, habitat characteristics and scavenger density can influence carcass detection as well (Wobeser and Wobeser, 2002). Furthermore, captured animals may not exhibit clinically obvious signs of poisoning (McDonald et al. 1998), making it difficult to assess whether testing for rodenticide exposure is warranted. For these reasons, it is likely that both the mortality and morbidity of non-target wildlife to SGARs is under-reported and DPR is correct to assume their dataset does not over-represent the hazard posed by these compounds.

DPR Response: Thank you for the comments.

Peer Reviewer Comment: In response to Charge Statement No. 3, the peer reviewer stated that based on DPR's analysis, it is evident that brodifacoum presents a hazard to non-target wildlife.

DPR conducted a thorough review of state-specific data that outlined an extensive range of affected non-target wildlife, including the endangered San Joaquin kit fox, bobcats, mountain lion, coyotes and several raptor species. From the DFG dataset, brodifacoum residue was reported in 68% of samples, indicating widespread exposure among wildlife. Recent studies that were not included in DPR's analysis reported brodifacoum in the majority of sampled fishers (Gabriel et al. 2012) and badgers (Quinn et al. 2012), providing additional evidence of the broad contamination of the terrestrial food chain.

Brodifacoum was implicated as a contributing factor in 13% of mortalities, and solely determined to be responsible for 9% of mortalities in DFG's dataset. Brodifacoum is listed as "extremely toxic" to both mammals and birds. The LD50 for dogs ranges from 0.25-1 mg/kg (Erickson and Urban, 2004). This level of toxicity is concerning for other canids, such as the San Joaquin kit fox, other foxes and coyotes. The LD50 is higher for cats, characterized as "highly toxic." Brodifacoum is considered to be "extremely toxic" to multiple avian species as well (Godfrey, 1986). The persistence of brodifacoum in the liver is high, with biphasic elimination characterized by a prolonged terminal phase and little or no metabolism of the parent compound. Studies on rats demonstrated an initial half-life of brodifacoum in the liver of 63 days, followed by 282 days (Hawkins et al. 1991). Brodifacoum persisted in possums that were administered sub-lethal doses for 9 months after exposure (Eason et al. 1996). In sheep, brodifacoum was detected 128 days after initial exposure (Laas et al. 1985).

While uncertainty exists to fully characterize the toxicodynamics of brodifacoum in non-target wildlife based on residues detected in the liver (Thomas et al. 2011), it is clear that these compounds occur throughout the terrestrial food chain, persist and contribute to wildlife mortality. Furthermore, the potential for sub-lethal effects following exposure to SGARs presents an additional hazard to non-target wildlife. Research has implicated the role of SGARs to negatively impact wildlife at sub-lethal levels, including studies on bobcats and mountain lions (Riley et al. 2007), owls (Mendenhall and Pank, 1980) and possums (Littin et al. 2002).

Based on DPR's analysis, bromadiolone presents a hazard to non-target wildlife. Bromadiolone residue was detected in 36% of tested samples, with a similar range of affected wildlife compared with brodifacoum. Furthermore, bromadiolone was detected in approximately 30% of the samples collected from fishers and badgers (Gabriel et al. 2012; Quinn et al. 2012), which were not included in DPR's analysis. Bromadiolone was implicated in less mortality compared with brodifacoum (3%). The toxicity to mammals is high, with LD50s ranging from approximately 8 mg/kg in the dog to > 25 mg/kg in cats (Erickson and Urban, 2004). Bromadiolone is less toxic to birds compared with brodifacoum, and categorized as "moderately toxic" (EPA 1998). Bromadiolone persists in the liver for long periods of time, with bi-phasic elimination characterized by a prolonged terminal phase. Studies on rats demonstrated an initial half-life of bromadiolone in the liver of 17 days, followed by 318 days (Hawkins et al. 1991). In a non-rodent study, bromadiolone persisted in sheep for over 8 months following a sub-lethal

exposure (Nelson and Hickling, 1994). Little or no metabolism of bromadiolone occurs. Similar to brodifacoum, uncertainty remains in the ability to translate liver residues into toxic effects for non-target wildlife. However, the widespread occurrence toxicity,
persistence and potential for sub-lethal effects of bromadiolone present a hazard to wildlife.

DPR Response: Thank you for your comments.

Peer Reviewer Comment: In response to Charge Statement No. 4, the peer reviewer stated that DPR is correct to infer that difethialone may present a hazard to non-target animals. Difethialone has a similar nonselective mode of action as the other SGARs and results in delayed mortality to the target pest, presenting an opportunity for secondary poisoning to predators or scavengers. While less persistent compared with brodifacoum, difethialone will remain in liver tissue for an extended time (Belleville 1991). Reports of difethialone positive residue spanned a similar range of affected wildlife species compared with brodifacoum and bromadiolone. Difethialone was detected in approximately 8% of samples analyzed in the DFG dataset. The lower percent of animals testing positive for difethialone likely reflects the current reduced use compared to brodifacoum and bromadiolone.

Difethialone is structurally similar to brodifacoum with a steep dose-response curve. The toxicity of difethialone to both birds and mammals is very similar to brodifacoum, and characterized as "extremely toxic" based on EPA's criteria (Erickson and Urban 2004). There is little or no metabolism of difethialone, with accumulation in the liver and bi-phasic elimination in the feces (Lechevin and Poche 1988). Based on the inherent toxicity, potential for accumulation and nonselective mode of action, it is probable that difethialone would pose similar hazards as brodifacoum if both compounds were to have equivalent use rates and patterns.

DPR Response: Thank you for your comments.

Peer Reviewer Comment: DPR cites the relatively high percentage of animals with residues above the most sensitive LD50 as part of their conclusion that difethialone may present a hazard. These percentages are summarized as the "Number above the most sensitive LD50" in Table 7 of the Memorandum. However, the values used to calculate the number of samples above the most sensitive LD50 (presented in Table 6 of the Memorandum), were derived from avian and mammalian feeding studies in which a controlled diet was administered to test animals and the LD50 determined by calculating dose to the test subject (Erickson and Urban 2004). The residues analyzed from DFG's dataset were limited to liver tissue, which is known to sequester SGARs. The comparison between LD50s from whole organisms in feeding studies to liver concentrations in animals is inappropriate. These values represent distinct data sets that have important differences in toxicokinetics and toxicodynamics. This issue applies to the other rodenticides summarized in Table 7 as well.

Difenacoum hazard to non-target wildlife: Given the lack of use information, field sampling and secondary poisoning data, it is more challenging to assess the potential hazard posed by difenacoum. The mode of action is similar to the other SGARs reviewed by DPR. Difenacoum accumulates and stores in the liver, with a similar bi-phasic pattern of elimination characterized by a longer terminal phase spanning several months (IPCS 1995). Difenacoum has similar toxicity compared with bromadiolone, categorized as "extremely toxic" and "moderately toxic" to mammals and birds, respectively. Based on its inherent toxicity, potential for accumulation and non-selective mode of action, difenacoum would be reasonably expected to pose a hazard to non-target wildlife if use were to increase.

DPR Response: Thank you for your comments.

Peer Reviewer Comment: In response to DPR's fifth charge statement (a), the peer reviewer noted that there is a clear need to understand the relationship between the level of SGARs in the liver and toxicity to non-target animals. The emphasis on incident data, clinical evaluation and occurrence of SGARs is important to identify the hazards posed by these compounds through secondary poisoning. However, a better understanding of the dose-response of SGARs among wildlife would help to characterize the risk of lethal and sub-lethal effects. Several obvious factors limit the ability to obtain this information across a broad spectrum of wildlife. However, the recognition of this data gap could be addressed in the Uncertainty section of DPR's Memorandum. Similarly, the inability to evaluate pesticide use patterns with toxicity and exposure, while acknowledged by DPR in the Uncertainty section, limits the capacity to provide a more detailed analysis of the non-target risks posed by SGARs.

DPR Response: Per the reviewer's comments, this was added to the Uncertainties section.

Peer Reviewer Comment:: In response to DPR's fifth charge statement (b), the peer reviewer noted that as a whole, DPR provided a sound and scientifically defensible approach to examine the hazards that are currently presented by brodifacoum and bromadiolone, or that may be posed by difethialone and difenacoum, to non-target wildlife, particularly in light of the challenges inherent to wildlife surveillance.

DPR Response: Thank you for all of your comments.