



# Department of Pesticide Regulation



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## MEMORANDUM

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SUBJECT: SECOND GENERATION ANTICOAGULANT RODENTICIDE ASSESSMENT

In a July 2011 memorandum, the California Department of Fish and Game (CDFG) requested that the Department of Pesticide Regulation (DPR) designate all second generation anticoagulant rodenticides as California restricted materials. This paper represents DPR's assessment, based on available data, of the potential and actual risk to non-target wildlife from second generation rodenticides.

### **Executive Summary**

Commensal mice and rats pose a significant economic and health risk to people. The rodenticides that are utilized to control them need to be efficacious while being relatively safe for humans, pets, and non-target wildlife. Rodenticides currently registered for use in California fall into three categories: first generation anticoagulant rodenticides (chlorophacinone, diphacinone and warfarin), second generation anticoagulant rodenticides (brodifacoum, bromadiolone, difenacoum, and difethialone), and non-anticoagulant rodenticides (bromethalin, cholecalciferol, and zinc phosphide).

Compared to first generation rodenticides, second generation anticoagulant rodenticides are considered to be more effective as they only require a single feeding and no resistance has been reported. Based on animal LD<sub>50</sub>s, second generation anticoagulant rodenticides have significantly longer half-lives in target and non-target wildlife, and are more toxic to birds and mammals.

DPR analyzed wildlife incident and mortality data between 1995 and 2011, and rodenticide use and sales data between 2006 and 2010. The data indicate that exposure and toxicity to non-target wildlife from second generation anticoagulant rodenticides is a statewide problem. In addition, the data suggest that the problem exists in both urban and rural areas. Research data from various locations throughout California indicate that exposure is occurring in many taxa and in various ecosystems (urban, suburban, rural, and natural/wild areas). While the data show exposure, they do not link specific uses, or location of use of second generation anticoagulant rodenticide (i.e., indoors or outdoors, homeowners or professionals) to exposure.

Of the 492 animals analyzed between 1995 and 2011, approximately 73% had residues of at least one second generation anticoagulant rodenticide. Brodifacoum residues were found in



approximately 69% of the 492 animals, and brodifacoum was likely involved in 13% of animal mortalities. Bromadiolone residues were found in approximately 37% of the animals analyzed, and bromadiolone was likely involved in approximately 3% of animal mortalities. Difethialone residues were found in approximately 8% of the animals analyzed. Due to its relatively new entrance into the marketplace, animals were not analyzed for difenacoum residues. While no animal mortalities can be directly attributed to difethialone or difenacoum, based on half-life and toxicity data, these two chemicals appear to be similar to brodifacoum and bromadiolone. Animals that tested positive for second generation rodenticides include bobcats, mountain lions, coyotes, foxes, skunks, hawks, crows, and owls.

The data also show that exposure of wildlife to second generation anticoagulant rodenticides can lead to sub-lethal effects. The sub-lethal effects reduce the fitness of wildlife at a time when wildlife are already meeting numerous challenges. Riley et al's (2007) study of bobcats is an example of the sub-lethal effects of rodenticides. The bobcats died due to *Toxoplasma gondii* mange. Mange was not previously known as a significant pathogen in wild felids. However, exposure to rodenticides appears to have contributed to the disease process, and hence, the mortality of the bobcats.

Based on the data reviewed, DPR finds that the use of second generation rodenticides presents a hazard related to persistent residues in target animals resulting in impacts to non-target wildlife.

### **Background**

Commensal mice and rats pose a significant economic and health risk to people, as they can damage homes, destroy crops, contaminate food, and directly spread eleven diseases (Center for Disease Control (CDC, 2011(b)) and indirectly spread fifteen diseases (CDC, 2012(a)) that threaten people's health and lives. Therefore, controlling them is considered a priority.

Rodenticides are pesticides that are designed to kill rodents, including mice and rats. For the purposes of this document, rodenticides will be divided into anticoagulant rodenticides (first and second generation) and non-anticoagulant rodenticides (including bromethalin, cholecalciferol, and zinc phosphide). Strychnine will not be discussed as its only labeled use is for below-ground gopher control.

First generation anticoagulant rodenticides - chlorophacinone, diphacinone, and warfarin - were developed and marketed beginning in 1950. However, by the 1970's, resistance to warfarin was noted in Norway rats, roof rats, and mice in Europe and North America. The warfarin-resistant strains of mice and rats prompted the development of second generation anticoagulant rodenticides, including brodifacoum, bromadiolone, difethialone, and difenacoum. Brodifacoum was developed in 1975, registered with the United States Environmental Protection Agency (U.S. EPA) in 1979, and registered with DPR in 1983. DPR first registered bromadiolone in 1982. The remaining two second generation rodenticides are relatively new. DPR first registered difethialone in 1997 and difenacoum in 2008.

Both first and second generation anticoagulant rodenticides are vitamin K antagonists that cause mortality by blocking an animal's ability to produce several key blood clotting factors. The result is a lag time between ingestion and death. The chemicals are likely to be additive in their effect (Gabriel et al (2012) and Riley et al (2007)), and can be treated with vitamin K (Merck Sharp & Dohme Corp (2011)). However, they differ in several key ways. First generation anticoagulant rodenticides require consecutive days of intake to accumulate a lethal dose and if the animal survives or doesn't like the taste or effects, it may develop bait shyness. If an animal consumes an anticoagulant rodenticide is eaten by a predator, the predator can become affected by the rodenticide (Townsend et al, 1984). However, the ability of first generation rodenticides to bioaccumulate in target and non-target animals is considered low (Eason and Ogilvie, 2009). The half-life (the amount of time it takes a substance to reduce its concentration by half) of most first generation anticoagulants in both target and non-target wildlife is generally hours to days, compared to the half-lives of second generation anticoagulants which are generally days to months. See Table 1 (below).

Second generation anticoagulant rodenticides have the same mechanism of action, but they have a higher affinity for the target enzyme (epoxide reductase enzyme), the ability to disrupt the vitamin K(1)-epoxide cycle at more points, and significantly longer half-lives in blood and liver (Watt et al, 2005) than first generation anticoagulant rodenticides. In general, rodents require only one feeding of bait to receive a lethal dose, although bromadiolone and difenacoum may require multiple feedings. Because it takes several days for the rodent to die, animals often eat multiple doses, allowing for super-lethal concentrations of the rodenticide to accumulate in its body. Second generation anticoagulant rodenticides become established in the animal's liver, with liver half-lives of four months to a year. If an animal that consumes a second generation anticoagulant rodenticide is eaten by a predator, the predator can become affected by the rodenticide. Because of their long half-lives, these rodenticides bioaccumulate in non-target wildlife (Annex I- Norway, 2007). See Table 1 (below).

The three non-anticoagulant rodenticides belong to three different chemical classes and differ from each other in their modes of action. Bromethalin is a neurotoxin that causes increased intracranial pressure and depending upon the dose, vomiting, seizures, paralysis, and death. Cholecalciferol is a sterol of vitamin D that, when converted in the liver into the active form, causes renal failure, cardiac abnormalities, hypertension, central nervous system depression, and gastric system distress (anorexia, vomiting, and constipation). Zinc phosphide is an inorganic rodenticide that converts to phosphine gas in the stomach, causing gastrointestinal distress (including vomiting and pain), hypotension, and cardiovascular collapse. See Table 1 (below) for the half-lives of rodenticides in the blood and liver of rats.

Table 1. Half-life (in days) of a single dose of rodenticides in the blood and liver of rats<sup>1, 2</sup>.

Class of Rodenticide	Rodenticide	Dose (mg ai/kg)	Half-life (in days) in Blood	Half-life (in days) in Liver
Second Generation Anticoagulant Rodenticides	Brodifacoum	0.02 to 0.35	6.5 to 91.7 <sup>7</sup>	113.5 <sup>3</sup> to 350
	Bromadiolone	0.2 to 3.0	1.0 to 2.4	170 to 318
	Difenacoum <sup>4</sup>	1.2	NA	118
	Difethialone	0.5	2.3	126
First Generation Anticoagulant Rodenticides	Chlorophacinone	4 to 5	0.4	Less than 2
	Diphacinone	0.32	NA	Between 2 and 3 <sup>1, 3</sup>
	Warfarin	NA <sup>9</sup> , 1 <sup>3</sup>	0.7 to 1.2 <sup>1</sup>	7 <sup>1</sup> to 26.2 <sup>3</sup>
Non-anticoagulant Rodenticides <sup>2</sup>	Bromethalin <sup>5</sup>	NA <sup>9</sup>	5.5	NA
	Cholecalciferol <sup>6</sup>	NA <sup>9</sup>	1	~19 <sup>8</sup>

- 1 Data summarized from Erickson and Urban, 2004, except where noted.
2. Data is not available for zinc phosphide, so it is not included on the chart.
3. Fisher et al, 2003.
4. U.S. EPA, 2007.
5. Spaulding and Spannring, 1988.
6. Marrow, 2001.
7. Vandenbroucke et al, 2008.
8. Body half-life (instead of liver half-life).
9. NA is defined as Not Available.

In 1999, CDFG requested that DPR place pesticide products containing the second generation anticoagulant rodenticide brodifacoum into reevaluation based on concerns regarding adverse effects to non-target wildlife. (Reevaluation is a process that allows DPR to evaluate the human health and environmental impacts of currently registered pesticide products.) After evaluating the data on file, DPR presented an issue paper recommending a number of mitigation measures and proposed that rodenticide baits containing brodifacoum, bromadiolone, and difethialone (difenacoum was not yet registered) be restricted to indoor structural use only. However, based on comments from representatives of the pest control industry expressing concern over the restriction, including comments from food processors noting that federal law requires rodent control to take place outside the building, DPR reconsidered its proposal.

DPR then became aware that the U.S. EPA was conducting risk assessments on numerous rodenticides. DPR decided to focus its reevaluation in coordination with U.S. EPA. In 2004, U.S. EPA (listed as Erickson and Urban, 2004) completed its *Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: a Comparative Approach*. In May 2008, U.S. EPA announced its final *Risk Mitigation Decision for Ten Rodenticides (RMD)*. At the time, all ten rodenticides came in various bait forms, including loose grains, pellets, and place packs, and only required the use of a bait station if the product could not be applied in locations out of reach of children. Most second generation anticoagulant rodenticides were labeled for use to

control rats and mice in and around homes, industrial, commercial, agricultural and public buildings, transport vehicles, and similar structures in urban areas. In addition to being labeled to control commensal rodents around homes, industrial sites, etc., first generation anticoagulants were labeled for agricultural uses, below-ground mole and pocket gopher control and vole control. While both first and second generation anticoagulant rodenticide were labeled for the residential marketplace, second generation anticoagulant rodenticides had the bulk of the residential market share.

The RMD describes U.S. EPA's risk mitigation decision for rodenticide products containing the following ten active ingredients: brodifacoum, bromadiolone, bromethalin, chlorophacinone, cholecalciferol, difenacoum, difethialone, diphacinone (and its sodium salt), warfarin (and its sodium salt), and zinc phosphide. The RMD includes two major components: (1) reducing children's exposure to rodenticide products used in the home, and (2) reducing wildlife exposures and ecological risks. To minimize children's exposure to rodenticide products used in homes, U.S. EPA's RMD requires that all rodenticides intended for use above ground by residential consumers be sold as solid formulations with a bait station. To reduce wildlife exposures and ecological risks, U.S. EPA imposed sales, package size, and use site restrictions to reduce the availability of second generation anticoagulant products to the residential consumer market. The RMD also requires the use of bait stations for most outdoor, above-ground uses of the ten rodenticides.

The terms and conditions of sale/distribution specified in the RMD and in U.S. EPA's notice of registration/reregistration prohibit the sale of second generation anticoagulant rodenticides in stores oriented towards residential consumers such as grocery, drug, hardware, home improvement stores, and other standard retail outlets. Sale and distribution of the products were restricted to agricultural, farm, and tractor stores or directly to pest control operators and other professional applicators. In addition, according to U.S. EPA's RMD, second generation anticoagulant products can only be sold in packages that contain eight or more pounds of bait. Products containing eight to sixteen pounds of bait are labeled only for use inside and within 100 feet of agricultural buildings and man-made agricultural structures vulnerable to rodent infestations. These products cannot be used in and around homes and residential sites. Products labeled for 16+ pounds of bait can be used in and within 100 feet of man-made structures (including homes and other residential areas) that are vulnerable to rodent infestations. The RMD initially restricted use to within 50 feet from buildings, but in a U.S. EPA memo dated March 14, 2012, the distance for all non-homeowner rodenticide products was increased to 100 feet and the definition of "building" was expanded to include man-made structures such as trash receptacles which are often placed farther than 50 feet from buildings. As stated above, these larger size quantities of second generation rodenticides are intended for distribution and sale at agricultural, farm, and tractor stores or directly to pest control operators and other professional applicators. The intent is to remove the product from general consumer access, while still having the products available to poultry and livestock producers and professional users, such as licensed pest control applicators. However, in California, numerous homeowners

live on the urban/rural edge and in rural areas on “ranchette” style properties (one to five acres of land per home). Due to the location and size of their property, people living in these areas, including ranchette owners, may shop at farm stores for supplies. Under current federal requirements, such individuals could purchase and use the 8 to 16 pound plus quantities of second generation anticoagulant rodenticides, even though they are not a “professional pesticide user.”

It is also important to note that not all second generation anticoagulant registrants complied with U.S. EPA’s mitigation measures. Six second generation anticoagulant products, targeted for the residential consumer market, are still registered for sale in California to residential consumers in grocery, drug, hardware, home improvement stores, and other standard retail outlets. On November 2, 2012, U.S. EPA took steps under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to cancel these noncompliant products by issuing a draft Notice of Intent to Cancel and convening a FIFRA Scientific Advisory Panel (SAP) at the end of November 2012. On February 5, 2013, the U.S. EPA issued a “Notice of Intent to Cancel Registrations of, and Notice of Denial of Applications for, Certain Rodenticide Bait Products”. However, to date, the outcome of these federal actions has not been determined. In addition, U.S. EPA existing stocks provisions for all consumer-use second generation anticoagulant rodenticides allow continued sale of such products from consumer oriented retail stores until supplies are exhausted.

A review of current California registered rodenticide labels shows that first generation anticoagulant and non-anticoagulant rodenticides are available to, and for use by, residential consumers (only in packages  $\leq$  1 pound of bait) to control rats and mice indoors and outdoors within 50 feet of homes or buildings. These consumer based products must be in a block/solid formulation, and be sold with, and used in, a bait station. First generation anticoagulant and non-anticoagulant products geared towards professional users ( $\geq$ 4 pounds of bait) can be used in and within 100 feet of buildings (including residential buildings) and inside of transport vehicles. For these products, bait stations are required for all outdoor, above-ground uses and indoors where children, pets, or non-target wildlife may be exposed. Some of these products are also labeled for baiting of rat burrows.

DPR also registers first generation anticoagulant and non-anticoagulant rodenticides that are labeled for use outdoors for manual below-ground burrow baiting to control pocket gophers and moles. Rodenticides containing diphacinone and chlorophacinone, and the non-anticoagulant rodenticide zinc phosphide have approved uses to control ground squirrels. Certain chlorophacinone, diphacinone, and zinc phosphide products can also be used in agricultural areas (orchards, fields, as well as landscaped areas such as parks and golf courses) and as tracking powders. However, all agricultural and tracking powder uses are designated as restricted use pesticides and can only be purchased and used by a California certified/licensed applicator or under their direct supervision. Liquid formulations of diphacinone sodium salt can be diluted and used indoors in non-residential areas by professional applicators.

DPR currently registers 72 end use products containing second generation anticoagulant rodenticides. As mentioned above, there are six second generation anticoagulant products targeted for use by homeowners in and around homes. Of the remaining 66 second generation anticoagulant rodenticide products currently registered, about half are labeled for use only inside and within 100 feet of agricultural buildings and other man-made agricultural structures. The other half are labeled for use inside and within 100 feet of man-made structures such as homes, food processing facilities, industrial and commercial buildings, trash receptacles, agricultural and public buildings, and transport vehicles and are intended for use by professional applicators (such as pest control operators, public health officials, federal, state, and municipal employees charged with rodent control). Certain products are also labeled for use in rodent burrows, alleys, and sewers. Bait stations must be used indoors when children, domesticated animals, or non-target wildlife may be exposed. Bait stations are required for all outdoor, above ground placements. Currently, there are no second generation anticoagulant rodenticides labeled for agricultural field use.

#### **Evaluated Data**

DPR considered data from multiple sources, including CDFG, private agencies and individuals, and available journal articles and other resources. Utilizing all of these resources, DPR was able to obtain information on almost 1,300 animals.

From that data set, DPR removed approximately half of the animals and multiple studies because the data were collected from outside California and placed the information in Appendix I. From the remaining 630 California animals, DPR removed an additional 41 animals (including 37 geese, 3 other birds, and 1 mammal) because all were related to a specific incident where chlorophacinone was used in artichoke fields after chopping or cut-back of artichoke plants. To address the problem, the product's label was amended to prohibit the application of chlorophacinone "for a period of 30 days before or after chopping or cut-back of artichoke plants." DPR also removed 26 rodents (including Norway (or brown) rats, roof (or black) rats, "rats" without a specified species, and all mice) as these are "target" animals. Four hawks, and a fox were also removed because only summary data were available (i.e., results on individual animals and for the individual rodenticides were not available. A snake (which contained difethialone) and a bobcat fetus (which contained residues of brodifacoum and diphacinone) were removed because there are no standards (i.e., LD<sub>50</sub> data on reptiles or bobcat fetus) against which to compare these animals. In addition, in each case only a single individual was available. When making scientific assessments, one usually wants data on more than one individual in order to assure that the data are not an anomaly. DPR placed summary information regarding the above animals in Appendix II. Also not included in DPR's main analysis are data on 58 fishers and 6 badgers that only recently became available. This new data is summarized in Appendix III.

Even though for scientific or timing reasons, DPR did not include the animals identified above in its main data analysis, the data still provide important information, and therefore, are summarized in Appendices I, II, and III.

DPR included all of CDFG's data in its main analysis, even though, in some cases, CDFG only reported animals that were positive for rodenticide residues (i.e., negative animals were excluded and the total number of animals analyzed was unknown). While including all of the CDFG data may result in an over representation of positive samples, DPR believes that the data provide value and do not over represent positive values for second generation anticoagulant rodenticides. Of the 492 animals included in DPR's analysis, 350 were from data sets that included both negative and positive samples. DPR compared the two data sets using statistical analysis (Chi-squared and Fisher's Exact with a level of significance of 0.05; using Preacher (2001)), and determined that the data sets (in regards to the second generation anticoagulant rodenticides, using the number of samples) are not significantly different. Therefore, DPR is comfortable including all CDFG data in its analysis.

### **Analysis**

The data included in this analysis were collected between 1995 and 2011, and came from the following: WildCare's data (WildCare (2011)), CDFG's data (CDFG (2011), CDFG (2012a), CDFG (2012b)), and McMillin et al (2008), Lima and Salmon's paper (Lima and Salmon (2010) and Lima and Salmon (2012)), Seth Riley's coyote data (Riley (2012)), and Riley et al's paper (Riley et al (2007)). The analysis includes 492 non-target animals (including 194 birds (primarily raptors) and 298 mammals (primarily San Joaquin kit fox, bobcats, mountain lions, coyotes, and foxes)).

The livers (and/or blood, in a few cases) of each animal were analyzed for at least six anticoagulant rodenticides. The animals were analyzed for the first generation - warfarin, chlorophacinone, and diphacinone - and second generation - brodifacoum, bromadiolone, and difethialone - anticoagulant rodenticides. In some cases, additional analyses were conducted, and those were reported where applicable. Two rodenticides not registered for use in California were also found, but will not be discussed. Those were coumachlor and pindone. In addition, because of its relatively recent entry into the rodenticide market, none of the 492 animals included in DPR's analysis were tested for difenacoum residues. Therefore, the lack of data showing difenacoum residues in animals is not indicative of a lack of toxicity.

Of the 492 non-target animals analyzed, approximately 75% had residues of one or more rodenticide, approximately 73% (359) had residues of at least one second generation anticoagulant rodenticide, and approximately 25% (124) were negative.

Brodifacoum residues were found in approximately 69% of the animals, bromadiolone residues were found in approximately 37% of the animals, and difethialone residues were found in approximately 8% of the animals. Of the animals that tested positive for at least one rodenticide,

approximately 98% had residues of at least one second generation anticoagulant rodenticide. Table 2 summarizes these results.

Table 2. Number (and percent) of the rodenticides among all animals (n=492) and among the positive animals (n=368)<sup>1</sup>.

Total	Number	Second Generation Anticoagulant Rodenticides			First Generation Anticoagulant Rodenticides		
Samples	492	359 (72.9%)			65 (13.2%)		
Positives	368	359 (97.6%)			65 (17.7%)		
Total	Number	Brodifacoum	Bromadiolone	Difethialone	Chlorophacinone	Diphacinone	Warfarin
Birds	194	124 (63.94%)	42 (21.7%)	10 (5.2%)	1 (0.5%)	5 (0.3%)	0 (0.0%)
Mammals	298	215 (72.2%)	141 (47.3%)	31 (10.4%)	17 (5.7%)	48 (16.1%)	4 (1.3%)
Total	492	339 (68.9%)	183 (37.2%)	41 (8.3%)	18 (3.7%)	53 (10.8%)	4 (0.8%)
Positives	368	339 (92.1%)	183 (49.7%)	41 (11.1%)	18 (4.9%)	53 (14.4%)	4 (1.1%)

1. Animals may be positive for more than one rodenticide.

This table indicates that exposure of non-target animals to second generation anticoagulant rodenticides far exceeds exposure to first generation anticoagulant rodenticides. In addition, brodifacoum residues were found in a large percentage of the animals (almost 70%).

Tables 3 and 4 show the bird and mammal data down to the species level.

Table 3. Number of each bird (n=194) species that was positive for a rodenticide, that was positive for a first or second generation anticoagulant rodenticide, and that was for each rodenticide<sup>1</sup>.

Species	n	Positive	2nd generation	Brodifacoum	Bromadiolone	Difethialone	1st generation	Chlorophacinone	Diphacinone	Warfarin
American Crow	1	1	1	1	0	0	0	0	0	0
American Kestrel	6	5	4	3	0	1	1	0	1	0
Bald Eagle	1	1	0	0	0	0	1	0	1	0
Barn Owl	49	29	29	28	15	2	0	0	0	0
Black Crowned Night Heron	1	0	0	0	0	0	0	0	0	0
Brown Pelican	2	0	0	0	0	0	0	0	0	0
Burrowing Owl	1	0	0	0	0	0	0	0	0	0
Canada Goose	1	0	0	0	0	0	0	0	0	0
Cooper's Hawk	17	14	14	14	3	0	1	0	1	0
Dark eyed junco	1	0	0	0	0	0	0	0	0	0
Golden Eagle	11	8	8	8	0	0	0	0	0	0
Great Horned Owl	21	17	17	16	8	1	1	0	1	0
Gull (any)	3	0	0	0	0	0	0	0	0	0
Hawk (unknown species)	1	1	1	1	1	1	0	0	0	0
Long-eared Owl	1	1	1	1	0	0	0	0	0	0
Northern Harrier	1	0	0	0	0	0	0	0	0	0
Prairie Falcon	1	0	0	0	0	0	0	0	0	0
Red-shouldered Hawk	22	17	16	16	8	1	0	0	0	0
Red-tailed Hawk	32	23	23	22	5	3	1	1	0	0
Sharp-shinned Hawk	9	6	6	6	0	1	0	0	0	0
Spotted Owl	2	1	1	1	0	0	0	0	0	0
Swainson's Hawk	1	1	1	1	0	0	0	0	0	0
Turkey Vulture	6	5	5	5	2	0	1	0	1	0
Western Screech Owl	3	1	1	1	0	0	0	0	0	0
Total	194	131	128	124	42	10	6	1	5	0

1. Animals may be positive for more than one rodenticide.

Table 4. Number of each mammalian (n=298) species that was positive for a rodenticide, that was positive for a first or second generation anticoagulant rodenticide, and that was for each rodenticide<sup>1</sup>.

Species	N	Positive	2nd generation	Brodifacoum	Bromadiolone	Difethialone	1st generation	Chlorophacinone	Diphacinone	Warfarin
Badger	3	1	0	0	0	0	1	1	1	0
Black Bear	3	3	3	3	3	0	2	2	2	0
Bobcats	41	36	35	31	26	11	15	1	13	1
Coyotes	44	36	33	33	12	4	8	4	6	1
Deer	1	0	0	0	0	0	0	0	0	0
Gray fox	9	7	7	7	4	1	2	0	2	0
Mountain Lions	28	28	28	27	26	11	18	3	17	2
Pig (Feral)	1	1	0	0	0	0	1	0	1	0
Raccoons	6	4	4	4	4	0	1	0	1	0
Red fox	37	35	35	30	25	2	4	0	4	0
San Joaquin kit fox	110	76	76	70	35	2	7	6	1	0
Skunk (any)	7	5	5	5	4	0	0	0	0	0
Squirrel (any)	5	2	2	2	0	0	0	0	0	0
Virginia Opossum	3	3	3	3	2	0	0	0	0	0
Total	298	237	231	215	141	31	59	17	48	4

1. Animals may be positive for more than one rodenticide.

### Comparisons to Wildlife LD<sub>50</sub>s

A LD<sub>50</sub> is the dose (in mg/kg of body weight) of a chemical that a species consumes in a single dose that is lethal to 50% of the animals of that species tested. A LC<sub>50</sub> is the concentration (in parts per million (ppm) or as mg/kg of body weight/day) of a chemical that produces mortality in 50% of the animals to which it is exposed (normally in the air, water, or food) in a given period of time. U.S. EPA has established guidelines for the LD<sub>50</sub>s and LC<sub>50</sub>s.

Table 5. Descriptive toxicity categories for wildlife compared to the LD<sub>50</sub>s and LC<sub>50</sub>s.

Descriptive Term	Mammal and Avian LD <sub>50</sub>	Mammal and Avian LC <sub>50</sub>
Extremely Toxic	< 10 mg/kg	< 50 ppm
Highly Toxic	10 – 50 mg/kg	50 – 500 ppm
Moderately Toxic	50 - 500 mg/kg	500 – 1,000 ppm
Slightly Toxic	500 – 2,000 mg/kg	1,000 – 5,000 ppm
Relatively Non-Toxic	> 2,000 mg/kg	> 5,000 ppm

Based on these descriptive categories, a rodenticide that is “extremely toxic” is toxic to 50% of the animals of that species tested at <10mg/kg of the chemical. However, there can be an apparent difference in sensitivities in the LD<sub>50</sub>s between species and even individuals. For example, the most sensitive LD<sub>50</sub> for brodifacoum is 0.26 mg/kg, is in a mallard. However, the Ring-necked pheasant has an LD<sub>50</sub> of 10 mg/kg (Erickson and Urban, 20004).

To equilibrate all of the finding, the most sensitive LD<sub>50</sub>s were used. Table 6 lists the LD<sub>50</sub>s and the descriptive toxicities (based on the U.S. EPA’s Pesticide Assessment Guidelines) for the nine rodenticides for the most sensitive birds and mammals.

Table 6. Most sensitive LD<sub>50</sub> and descriptive toxicity<sup>1</sup> for birds and mammals for nine rodenticides<sup>2</sup>.

Type of Rodenticide	Rodenticide	Most sensitive LD <sub>50</sub> for Birds (in mg/kg)	Descriptive Toxicity for the most sensitive Birds LD <sub>50</sub>	Most sensitive LD <sub>50</sub> for Mammal (in mg/kg)	Descriptive Toxicity for the most sensitive Mammal LD <sub>50</sub>
Second Generation Anticoagulant Rodenticides	Brodifacoum	0.26	Extremely Toxic	0.13	Extremely Toxic
	Bromadiolone	138	Moderately Toxic	0.56	Extremely Toxic
	Difenacoum	66 <sup>3</sup>	Moderately Toxic	0.45 <sup>3</sup>	Extremely Toxic
	Difethialone	0.26	Extremely Toxic	0.29	Extremely Toxic
First Generation Anticoagulant Rodenticides	Chlorophacinone	>100	Moderately Toxic	0.49	Extremely Toxic
	Diphacinone	96.8 <sup>4</sup>	Moderately Toxic	0.2	Extremely Toxic
	Warfarin	620	Slightly Toxic	2.5	Extremely Toxic
Non-Anticoagulant Rodenticides	Bromethalin	4.6	Extremely Toxic	2.0	Extremely Toxic
	Cholecalciferol	>600	Slightly Toxic	5.5	Extremely Toxic
	Zinc phosphide	8.8	Extremely Toxic	26	Highly Toxic

1. From the EPA Pesticide Assessment Guidelines (U.S. EPA, 2011).
2. Data summarized from Erickson and Urban, 2004, except where noted.
3. U.S. EPA, 2007.
4. Rattner et al, 2011.

The data indicate that the second generation anticoagulant rodenticides brodifacoum and difethialone are extremely toxic to both birds and mammals. The second generation anticoagulant rodenticides bromadiolone and difenacoum are moderately toxic to birds, but extremely toxic to mammals.

It is important to note that LD<sub>50</sub> tests are run in a laboratory setting, where the animals are not subject to the need to forage, or to predation or pathogen pressures. Additionally, the LD<sub>50</sub> considers only one endpoint: mortality. Multiple studies (Eason et al (1996), Fisher (2009), and Naz et al (2011)) have shown that even sub-lethal doses can cause clotting, biochemical (including glucose and liver function markers), and physiological abnormalities (including

statistically significant decreased body weight, increased liver size, increased heart size, and increased kidney size), which could or did cause mortality in the laboratory setting.

Field and epidemiological studies can provide additional information about what happens in non-laboratory situations. Dowding et al (1999) analyzed brodifacoum concentrations in the livers of cats, rabbits, and birds found dead or euthanized on Motuihe Island following a Norway rat and house mouse eradication operation in August 1997. Three cats found dead had liver brodifacoum concentrations of 0.91 to 1.38 ppm. Five rabbits found dead on the island had liver concentrations of 0.05 to 2.01 ppm. Twenty-nine non-target birds (including ducks, raptors, and songbirds) that were found dead had liver concentrations of 0.12 to 2.31 ppm. The incidence of mortality 2 weeks after the eradication was 49% in the pukeko flock (order: Gruiformes; a coot) and 60% in the paradise shelduck flock (order: Anseriformes; a duck). It is likely, given their behavior and eating habits, that the rabbits and paradise shelduck directly consumed the bait, while the cats and raptors would most likely have consumed prey items that had consumed the bait. Depending upon the species, circumstances, and individual involved, the songbirds and pukeko may have directly consumed the bait and/or consumed prey that consumed the bait.

Riley et al (2007) found that all 19 of the bobcats that died due to severe notoedric mange were exposed to second generation anticoagulant rodenticides, with brodifacoum ranges from trace to 0.56 ppm. Morbidity or mortality due to notoedric mange had not previously been reported as a significant pathogen in wild felid. The study demonstrated that where the levels of second generation anticoagulant rodenticides were more than 0.05 ppm, the correlation to mange (and mortality) was “highly significant” with a p-value < 0.01.

In the laboratory, second generation rodenticides are also known to cause lethargy, shortness of breath, anorexia, bloody diarrhea, changes in behavior, potential heart damage, and tenderness of the joints (Cox and Smith (1992), Housenger and Melendez (2011), IPCS (2010), Littin et al (2000), Merck Sharp & Dohme Corp (2011), Munday and Thompson (2003), Naz et al (2011), Rahmy (1993), Shlosberg and Booth (2001), Valchev (2008), and Woody et al (1992)). Therefore, even sub-lethal exposure to anticoagulants may contribute to the ill thrift of the animal. U.S. Fish and Wildlife Service (2010) stated that, “Even in cases where the proximate cause of death has been identified as automobile strike, predation, or disease, toxicologists and pathologists have attained sufficient toxicological evidence to conclude that rodenticide-induced blood loss increased animal vulnerability to the proximate cause of death.”

The concentration of brodifacoum in the liver (which is in ppm), while not always an accurate reflection of the amount of brodifacoum ingested (which is in mg/kg), demonstrates exposure and when a necropsy is conducted, is often used in conjunction with everything else to assess the potential mortality based on the liver residues of the rodenticide. For example, Eason et al (1996) dosed the Common Brushtail Possum (*Trichosurus vulpecula*) with 0.1 mg/kg and found mean liver concentrations of 0.100 ppm 14 days after dosing, 0.109 ppm 63 days after the dosing, and 0.075 ppm 126 days after dosing. Fisher et al (2003) dosed rats with 0.1 mg/kg brodifacoum and

found the mean liver residue concentration after one week to be 1.27 ppm, after 18 weeks to be 0.59 ppm, and after 24 weeks to be 0.49 ppm. Additionally, Eason et al (1999) dosed pigs with brodifacoum in single dietary doses of 0.57 ppm, 0.96 ppm, and 1.94 ppm and then analyzed their livers on the fifth day. When the pigs consumed approximately 0.57 mg/kg, 0.96 mg/kg, and 1.94 mg/kg, the resulting brodifacoum concentration in the liver was 1.13 ppm, 1.08 ppm, and 1.05 ppm, respectively. If the whole body concentration of brodifacoum were analyzed, instead of just the concentration in the liver, the concentration would be significantly lower. Because the liver essentially collects the rodenticide, the liver is analyzed, which allows for the determination of exposure. However, because the liver collects the rodenticide, the rodenticide can be found at a higher concentration in the liver than in the animal at a whole.

Of the 492 animals included in this analysis, 368 (approximately 75%) had residues of at least one first and/or second generation anticoagulant rodenticide. Table 7 quantifies the number and percent of samples that had residues (including trace residues), those which had measurable (i.e., non-trace) residues, and those which had residues above the most sensitive LD<sub>50</sub>.

Table 7. Number (and percent) of animals that had anticoagulant rodenticide residues (including trace residues), had measurable (i.e., non-trace) residues, and that had anticoagulant levels above the most sensitive LD<sub>50</sub> (n=492)<sup>1</sup>.

Rodenticide		Avian	Mammal	Total
Any	Total Number of samples	194 (100%)	298 (100%)	492 (100%)
	Total Number with no residues <sup>2</sup>	63 (32.5%)	61 (20.5%)	124 (25.2%)
	Total Number of positive samples <sup>3</sup>	131 (67.5%)	237 (79.5%)	368 (74.8%)
Brodifacoum	Total Number with no residues <sup>2</sup>	70 (36.1%)	83 (27.9%)	153 (31.1%)
	Total Number of positive samples <sup>3</sup>	124 (63.9%)	215 (72.1%)	339 (68.9%)
	Number with measurable residues <sup>4</sup>	107 (55.2%)	199 (66.8%)	306 (62.2%)
	Number above the most sensitive LD <sub>50</sub> <sup>5</sup>	26 (13.4%)	85 (28.5%)	111 (22.6%)
Bromadiolone	Total Number with no residues <sup>2</sup>	152 (78.4%)	157 (52.7%)	309 (62.8%)
	Total Number of positive samples <sup>3</sup>	42 (21.6%)	141 (47.3%)	183 (37.2%)
	Number with measurable residues <sup>4</sup>	26 (13.4%)	111 (37.2%)	138 (28.0%)
	Number above the most sensitive LD <sub>50</sub> <sup>5</sup>	0 (0.0%)	38 (12.8%)	38 (7.7%)
Difethialone	Number with no residues <sup>2</sup>	184 (94.8%)	267 (89.6%)	451 (91.7%)
	Total Number of positive samples <sup>3</sup>	10 (5.2%)	31 (10.4%)	41 (8.3%)
	Number with measurable residues <sup>4</sup>	5 (2.6%)	4 (1.3%)	9 (1.8%)
	Number above the most sensitive LD <sub>50</sub> <sup>5</sup>	5 (2.6%)	4 (1.3%)	9 (1.8%)
Chlorophacinone	Number with no residues <sup>2</sup>	193 (99.5%)	250 (83.9%)	439 (89.3%)
	Total Number of positive samples <sup>3</sup>	1 (0.5%)	17 (5.9%)	18 (3.7%)
	Number with measurable residues <sup>4</sup>	0 (0.0%)	11 (3.7%)	11 (2.2%)
	Number above the most sensitive LD <sub>50</sub> <sup>5</sup>	0 (0.0%)	3 (1.0%)	3 (0.6%)
Diphacinone	Number with no residues <sup>2</sup>	189 (97.4%)	250 (83.9%)	439 (89.3%)
	Total Number of positive samples <sup>3</sup>	5 (2.6%)	48 (16.1%)	53 (10.8%)
	Number with measurable residues <sup>4</sup>	3 (1.5%)	17 (5.7%)	20 (4.1%)
	Number above the most sensitive LD <sub>50</sub> <sup>5</sup>	2 (1.0%)	10 (3.4%)	12 (2.4%)
Warfarin	Number with no residues <sup>2</sup>	194 (100.0%)	294 (98.7%)	488 (99.2%)
	Total Number of positive samples <sup>3</sup>	0 (0.0%)	4 (1.3%)	4 (0.8%)
	Number with measurable residues <sup>4</sup>	0 (0.0%)	2 (0.7%)	2 (0.4%)
	Number above the most sensitive LD <sub>50</sub> <sup>5</sup>	0 (0.0%)	0 (0.0%)	0 (0.0%)

1. Animals may be positive for more than one rodenticide.
2. The number of samples with no residues is the number of samples that did not have trace or measurable amounts in it. It can be added to the Number of Total Number of positive samples to get the Total Number of samples.
3. The samples that tested positive for a sample may have had trace (i.e., when the rodenticide is known to be present but its level is so low that it cannot be quantified) or measurable (i.e., when the amount of a rodenticide can be put into a number) amounts of the rodenticide. It can be added to the Number of samples with no residues to get the Total Number of samples.
4. The number of samples with measurable or quantifiable residues includes only the samples where the amount of a rodenticide can be put into a number (i.e., it does not include the trace detections). This is part of the Total Number of

positive samples, but does not include the trace samples (i.e., the Total Number of positive samples minus Number with measurable residues will equal the number with trace residues). For this reason, this number should not be added to any of the other categories.

5. The number of samples above the LD<sub>50</sub> includes only those samples that have measurable residues and that are above the most sensitive LD<sub>50</sub> (a measurable amount) for the species (listed in Table 6). This is part of the Number with measurable residues, but lacks those that are not above the LD<sub>50</sub> (i.e., the Number with measurable residues minus the Number above the most sensitive LD<sub>50</sub> will equal the number that fell between those that had measurable detections and those that were above the LD<sub>50</sub>). For this reason, this number should not be added to any of the other categories.

This table indicates that number of non-target animals that had second generation anticoagulant rodenticide residues (including trace residues), had measurable (i.e., non-trace) residues, and that had anticoagulant levels above the most sensitive LD<sub>50</sub> (n=492)<sup>1</sup> exceeds the numbers for first generation anticoagulant rodenticides. Brodifacoum residues were found in approximately 69% of samples and in those samples brodifacoum residues were above the most sensitive LD<sub>50</sub> approximately 23% of the time. Bromadiolone residues were found in approximately 37% of samples and in those samples bromadiolone residues were above the most sensitive LD<sub>50</sub> approximately 8% of the time. Difethialone residues were found in approximately 8% of samples and in those samples difethialone residues were above the LD<sub>50</sub> approximately 2% of the time. While liver residues above the LD<sub>50</sub> (or sometimes even below) indicates that some of these animals could have died due to the concentrations of the rodenticide seen in their liver, it is difficult to definitely correlate exposure to the cause of death of an individual, without evidence of coagulopathy at necropsy.

### Necropsies

Out of the 492 animals analyzed, 211 necropsies (including 124 birds and 87 mammals) were conducted. The 80 necropsies presented to DPR for evaluation were conducted by veterinarians (including both those with advanced training in pathology and those without advanced training in pathology) and non-veterinarians, and were assessed accordingly. The remaining necropsies were present in Lima and Salmon's and Riley et al's papers.

Multiple difficulties can arise when conducting a necropsy, including a freeze-thaw artifact, a decomposing body, and/or if predated body. In many cases, necropsies on animals with these problems were excluded or were assessed more carefully.

Of the 211 necropsies, 38 (approximately 19%) indicate that anticoagulant rodenticides contributed to or could be correlated to morbidity (i.e., disease), but were not the cause of death, or more information or analysis was needed to establish the cause of death. Thirty-three (33) of the necropsies (approximately 16%) indicate that anticoagulant rodenticides were likely a cause of death or the cause of death. Of the 33 cases where anticoagulant rodenticides were the most likely cause of death, second generation rodenticides were involved in 29 cases (approximately 14%). Specifically, brodifacoum was involved in 28 cases (approximately 13%), and brodifacoum was likely the sole or primary cause of death in 20 cases (approximately 9%).

Additionally, bromadiolone was involved in 7 cases (approximately 3%). Table 8 summarizes the results.

Table 8. Summary of the rodenticides identified as the likely cause of death (based on the analysis of the necropsies), the concentration(s) of the individual rodenticide(s), and the total rodenticides concentration in the liver of the animal.

Birds (n=124)	Mammals (n=87)	Number (n=211)	Primary Rodenticide(s) Involved	Rodenticide Concentration (ppm)	Total Rodenticide Concentration
9 (7.3%)	11 (12.6%)	20 (9.5%) <sup>1</sup>	Brodifacoum	Trace to 11.0	Trace to 11.0
4 (3.2%)	2 (2.3%)	6 (2.8%) <sup>2</sup>	Brodifacoum Bromadiolone	0.07 to 0.57 0.065 to 1.27	0.38 to 1.84
1 (0.8%)	0 (0.0%)	1 (0.5%) <sup>3</sup>	Bromadiolone	0.38	0.38
1 (0.8%)	1 (1.1%)	2 (1.0%) <sup>4</sup>	Brodifacoum Diphacinone	0.002 to 0.08 0.169 to 1.30	0.171 to 1.38
2 (1.6%)	0 (0.0%)	2 (1.0%) <sup>5</sup>	Diphacinone	Trace to 3.5	Trace to 3.5
0 (0.0%)	2 (2.3%)	2 (1.0%) <sup>6</sup>	Chlorophacinone	0.4 to 1.2	0.4 to 1.2
17 (13.7%)	16 (18.4%)	33 (15.6%) <sup>7</sup>	Total		

1. The 9 birds were a Cooper's Hawk, a Turkey Vulture, 2 Barn Owls, 2 Great Horned Owls, and 3 Golden Eagles. The 11 mammals were a mountain lion, an opossum, a red fox, an endangered San Joaquin kit fox, 2 bobcats, 2 fox squirrels, and 3 coyotes.
2. The 4 birds were 2 Barn Owls and 2 Great Horned Owls. The 2 mammals consisted of 2 mountain lions.
3. The bird was a Barn Owl.
4. The bird was a Barn Owl. The mammal was a coyote.
5. The 2 birds were a Bald Eagle and Turkey Vulture.
6. The 2 mammals were a coyote and a bobcat.
7. The 17 birds were a Bald Eagle, a Cooper's Hawk, 2 Turkey Vultures, 3 Golden Eagles, 4 Great Horned Owls, and 6 Barn Owls. The 16 mammals were an opossum, a red fox, an endangered San Joaquin kit fox, 2 fox squirrels, 3 bobcats, 3 mountain lions, and 6 coyotes.

Of the 29 necropsies where second generation anticoagulant rodenticides were the likely cause of death, the overall levels of second generation anticoagulant rodenticides ranged from trace to 11.0 ppm. In the 20 cases where brodifacoum was the primary or sole compound that caused mortality, brodifacoum residues ranged from trace to 11.0 ppm.

### Animal Information, Diet, and Habitat

The Migratory Bird Treaty Act of 1918 prohibits the take of native birds (including killing or causing the death of a bird) without a permit. Additionally, Bald and Golden Eagles are further protected by the Bald and Golden Eagle Protection Act of 1962. The majority of the birds analyzed in this paper are carnivores that are likely exposed to rodenticides either by secondary or tertiary exposure. The Barn Owl and the Great Horned Owl are nocturnal raptors. The Barn Owl prefers to hunt in open country and along the edges of woods (in rural and natural areas),

but also lives in urban and suburban areas. They primarily eat rodents, but will also eat other small mammals, birds, and invertebrates (Rocha et al (2011) and Pezzo and Morimando (1995)). Great Horned Owls prefer wooded (natural) and forested areas, but will live in natural, suburban, rural and urban areas. They primarily eat small to medium mammals (such as rabbits, and rodents), but will also eat larger mammals, birds (including other raptors), reptile, amphibian, and fish (Marti and Kochert (1996)).

Bald Eagles, Cooper's Hawks, Golden Eagles, Red-shouldered Hawks, Red-tailed Hawks, and Turkey Vultures are diurnal raptors. Bald Eagles tend to live among trees near water, and prefer natural or rural areas (Guinn (2004)). They primarily eat fish, but will also eat carrion, mammals, avian (including other raptors), reptiles, amphibians, and invertebrates (Peterson (1986)). Cooper's Hawks are agile fliers that fly through thick cover (including trees, vegetation, and buildings) to catch its prey. They prefer wooded and forested areas, but live in urban, suburban, rural, and natural areas. They primarily prey upon on birds, but will also eat mammals (Roth and Lima (2003)). Golden Eagles prefer nesting on mountains and hunting in open areas, such as rural areas (non-agricultural) and natural areas (Carrette et al (2000) and Marzluff et al (1997)). They primarily eat rabbits and squirrels, but will take prey weighing 1 to 15 pounds, including mammals, birds (including other raptors), reptiles, amphibians, fish, insects, and carrion (Bloom and Hawks (1982), and Steenhof and Kochert (1998)). The Red-shouldered Hawk prefers to live in woodlands (natural areas), especially near rivers or swamps, but will live in suburban and rural areas. They primarily prey upon small mammals (especially rodents), but will also consume reptiles, amphibians, birds, and crayfish (Jacobs and Jacobs (2002)). The Red-tailed Hawk prefers to live in open (rural or natural) areas, but also live urban and suburban areas. They primarily prey upon rodents, but will also consume other mammals (including predators), birds (including other raptors), reptiles, amphibians, and insects (Gatto et al (2005), and Steenhof and Kochert (1998)). Turkey Vultures prefer open areas, such as rural and natural areas. Their diet is almost exclusively composed of carrion, including small and large mammals, birds, reptiles, and fish (Hiraldo et al (1991a) and Hiraldo et al (1991b)).

Some of the mammals analyzed in this paper included bobcats, mountain lions, coyotes, red foxes, San Joaquin kit foxes, fox squirrels, opossum, and skunks. Bobcats and mountain lions are solitary animals and strict carnivores (normally only eat meat). Mountain lions tend to found primarily in rural and natural areas. A mountain lion's diet is primarily composed of ungulates (primarily deer), although they will also eat rodents, insects, and predators (including coyotes), depending upon location, season, and abundance (Blakenship (1995), Iriarte et al (1990), and Riley et al (2007)). They are most likely to be exposed to rodenticides by tertiary (i.e., the animal eats an animal that ate an animal that ate the rodenticide) exposure, although secondary exposure is possible. Bobcats prefer woodland (natural areas), but will live in rural areas, in some suburban areas, as well as on the edges of urban areas. They primarily consume rodents and rabbits, although they will also consume insects, reptile, and larger prey (including deer), depending upon availability, season, and preference (Blakenship (1995) and Litvaitis (1981)). They are most likely to be exposed by secondary exposure, although tertiary exposure is possible.

Coyotes are a medium sized generalist predator that can live in urban, suburban, rural, or natural environments. They primarily eat small mammals (such as rodents, rabbits, and squirrels), but will also eat birds, snakes, deer, seed, and fruit (Blakenship (1995)). There are three red fox species in California: the Sacramento Valley red fox, the Sierra Nevada red fox, and the non-native red fox (Sacks et al (2010)). Although the subspecies can differ in distribution, appearance, and behavior, the red fox will, in general, live in urban, suburban, rural, and natural environments. They are crepuscular animals that primarily eat rodents, but their diet also includes birds, insects, other mammals (including other predators), small deer, fish, fruit, carrion, and refuse (Lariviere and Pasitschniak-Arts (1996), and Papakosta et al (2010)). The San Joaquin kit foxes are a small (approximately five pounds) canid that is federally listed as endangered. They are only found in the San Joaquin Valley and Central Coast of California, but they live in urban (including downtown Bakersfield), suburban, rural, and natural areas. They primarily eat rodents (including kangaroo rats), rabbits, and squirrels, but will also consume reptiles, insects, birds, carrion, fruit, and refuse (Frost (2005)), McGrew (1979)) and Warrick et al (2007)), depending upon season, availability, and location. Coyotes and foxes most likely ingest rodenticides secondarily (by ingesting a rodent or squirrel), although they could be exposed via tertiary exposure or by directly consuming it.

Fox squirrels prefer forested areas, but can be found in urban, suburban, rural, and natural environments. They consume tree seeds, tree buds, tree flowers, bird eggs, and mushrooms (Lee et al (2001)) and Koprowski (1994)). They are most likely exposed to rodenticides through direct ingestion. Virginia opossums are a marsupial. They can live in urban, suburban, rural, and natural environments. Opossums are opportunistic omnivores, eating insects, plants, fruit, mammals (dead or alive), birds, reptiles, and refuse (McManus (1974)). They are most likely exposed to rodenticides by direct consumption or secondary exposure. Skunks live in urban, suburban, rural, and natural areas. They are crepuscular omnivores that eat primarily insects, but will also eat vertebrates, carrion, eggs, fruit, leaves, grains, nuts, and refuse (Kasparian et al (2002) and Wade-Smith and Verts (1982)). They most likely ingest rodenticides by secondary exposure, but may also be exposed by tertiary exposure or direct ingestion the rodenticide.

### **Location & Land Use**

Of the 492 animals, counties were provided for 491 of them and more precise locations (i.e., urban, rural, natural/wild area based on population and/or land use) were provided for 248 animals. DPR looked at the location where each of the animals analyzed were found to determine whether the animals were found in predominately urban, rural, or natural (wild areas) settings.

The 492 animals came from at least 35 California counties, including Alameda, Colusa, Contra Costa, El Dorado, Fresno, Glenn, Kern, Kings, Los Angeles, Madera, Marin, Mendocino, Merced, Monterey, Napa, Orange, Placer, Riverside, Sacramento, San Benito, San Bernardino, San Diego, San Francisco, San Joaquin, San Luis Obispo, San Mateo, Santa Clara, Santa Cruz, Sonoma, Solano, Stanislaus, Sutter, Tulare, Ventura, and Yolo.



Table 9. Number of raptors analyzed that had anticoagulant rodenticide residues (including trace residues) by region from 2006 to 2009 (n = 96 raptors)<sup>1,2</sup>.

Region	Number of samples analyzed	Second Generation Anticoagulant Rodenticides			First Generation Anticoagulant Rodenticides		
		Brodifacoum	Bromadiolone	Difethialone	Chlorophacinone	Diphacinone	Warfarin
San Diego County	53	49 (92.4%)	22 (41.5%)	8 (15.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Central Valley	43	25 (58.1%)	5 (11.6.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)
Total	96	75 (78.1%)	28 (29.2%)	8 (8.3%)	1 (1.0%)	0 (0.0%)	0 (0.0%)

1. Animals may be positive for more than one rodenticide.
2. Data differs from Lima and Salmon’s Table 3.

In San Diego County, there was no statistical difference between the percentage of animals with residues of brodifacoum, bromadiolone, and difethialone in rural areas (as defined by population) and urban areas (using Preacher (2001)), even though bromadiolone residues were found in a higher percentage of urban samples than in the rural samples. See Tables 10 and 13, below.

Table 10. Number of raptors analyzed that had anticoagulant rodenticide residues (including trace residues) by population density/land use within San Diego County from 2006 to 2009 (n=53 raptors)<sup>1,2</sup>.

Population Density	Number of Animals	Brodifacoum	Bromadiolone	Difethialone
Urban	17	16 (94.1%)	9 (52.9%)	3 (17.6%)
Unknown	1	0 (0.0%)	0 (0.0%)	0 (0.0%)
Rural	35	33 (94.3%)	13 (37.1%)	5 (14.3%)
Total	53	49 (92.5%)	22 (41.5%)	8 (15.1%)

1. Samples may be positive for more than one rodenticide.
2. None of the samples were positive for a first generation anticoagulant rodenticide.

San Joaquin kit foxes are federally listed as an endangered species and state listed as threatened. According to the U.S. Fish & Wildlife Services, ““Endangered” means a species is in danger of extinction throughout all or a significant portion of its range.” Although the number San Joaquin kit fox living in Bakersfield *might* be as high as 400 individuals, this number has not been deemed sufficient to keep them from going extinct, especially since “a century ago, more than 12,000 if the foxes roamed the San Joaquin Valley (Cypher (2010)).”

In their “5-Year Review: Summary and Evaluation” the U.S. Fish and Wildlife Service (2010) found that

Pesticides, and specifically rodenticides, pose a threat to kit fox through direct or secondary poisoning. For example, kit fox may be killed if they ingest rodenticide

in a bait application, or if they consume rodents that have consumed bait... Secondary exposure to SGARs is particularly problematic due to the high toxicity of the compounds and their long persistence in body tissues. For example, brodifacoum, a common SGAR, is persistent in tissue, bioaccumulates, and appears to impair reproduction... Even in cases where the proximate cause of death has been identified as automobile strike, predation, or disease, toxicologists and pathologists have attained sufficient toxicological evidence to conclude that rodenticide-induced blood loss increased animal vulnerability to the proximate cause of death (USEPA 2008)... the Service expects that effects of rodenticide exposure could have substantial population level effects where exposure is present, especially where kit fox populations are small and where they rely on target species, such as ground squirrels and murid rodents, for prey.

DPR found that of the samples, approximately 73% were positive for second generation anticoagulant rodenticides, and out of 110 San Joaquin kit foxes that were sampled, approximately 64% of the animals were positive for brodifacoum and approximately 33% were positive for bromadiolone, which includes 13 kit fox in the relatively isolated Lokern area (an isolated area where only animals had residues for bromadiolone). For instance, in 2009, of the 4 animals that were analyzed, all 4 were found in Bakersfield and 3 of the 4 had brodifacoum residues. And, in 2011, of the 4 animals that were analyzed, all 4 were found in Kern County and all 4 had brodifacoum and bromadiolone. Since 2009, 7 of the 8 animals (87.5%) have had second generation rodenticides, specifically brodifacoum. Additionally, there was likely at least 1 mortality that was most likely caused by brodifacoum. Of the approximately 400 animals in Bakersfield, this indicates that between 293 and 350 might have residues for a second generation rodenticide. Based on the analysis by U.S. Fish and Wildlife Service, the exposure to second generation anticoagulant rodenticides can cause take, including mortality, which could have “substantial population level effects” on an endangered species that is “in danger of extinction.”

Additionally, of the 120 animals analyzed from the San Joaquin kit fox data (CDFG (2011) and CDFG (2012b)), including 110 San Joaquin kit fox, 1 badger, 1 bobcat, 2 coyotes, 2 skunks, and 4 red foxes, approximately 80% of the animals from Bakersfield (an urban area) had residues of brodifacoum. Only 30% of the animals had residues of brodifacoum from “other locations” in Kern, San Benito, San Luis Obispo, and Tulare counties (which could include urban, suburban, rural, agricultural, and/or natural areas) and none of the animals from Lokern (a 40,000 acre natural area, designed to provide quality brush scrub habitat for threatened and endangered plants and animals) had residues of brodifacoum. While, one animal collected from Lokern (in 2007) was positive for bromadiolone, there was a significant difference between the number of animals in the urban and rural areas that were positive for brodifacoum and bromadiolone. See Tables 11 and 13, below.

Table 11. Number of animals analyzed that had anticoagulant rodenticide residues (including trace residues) by location (land use and County) from 1999 to 2011 (n=120)<sup>1,2</sup>.

Location (Land use/type)	County	Number	Second Generation Anticoagulant Rodenticides			First Generation Anticoagulant Rodenticides	
			Brodifacoum	Bromadiolone	Difethialone	Chlorophacinone	Diphacinone
Bakersfield (Urban)	Kern	75	60 (80.0%)	29 (38.7%)	0 (0%)	6 (8.0%)	2 (2.7%)
Unknown	Kern	10	7 (70.0%)	8 (80.0%)	2 (20.0%)	1 (10.0%)	0 (0%)
Other <sup>3</sup>	Various	20	6 (30.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Lokern (Natural Area)	Kern	15	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)
Total		120	73 (60.8%)	38 (31.7%)	2 (1.7%)	7 (5.8%)	2 (1.7%)

1. Samples may be positive for more than one rodenticide.
2. None of the samples were positive for warfarin.
3. "Other" includes areas in Kern, San Benito, San Luis Obispo, and Tulare counties which could include urban, suburban, rural, agricultural, and/or natural areas.

Multiple studies have been conducted on coyotes, bobcats, and mountain lions, in Los Angeles and Ventura Counties, in urban and rural areas, including in the Santa Monica Mountains National Recreation Area (SMMNRA). The SMMNRA preserve is over 150,000 acres in the Santa Monica Mountains, between the Pacific Ocean and the inland valley. It contains many individual parks and open spaces, and is administered by the National Park Service (NPS), in conjunction with multiple state and local agencies and groups. While some parks and spaces within the park do not use second generation anticoagulant rodenticides, at least one facility in the NPS uses bromadiolone inside tamper-proof boxes (Miller, 2012).

Of the 28 mountain lions found in eight counties that were tested between 1997 and 2011, 100% tested positive for a second generation rodenticide, approximately 96% tested positive for brodifacoum, 93% tested positive for bromadiolone, and 39% tested positive for difethialone (almost all of the mountain lions were positive for more than one rodenticide). In their study of mountain lions and bobcats in the Santa Monica Mountains (including in the SMMNRA) and Simi Hills of Los Angeles and Ventura Counties, Riley et al (2007) found that mountain lions were "less urban-associated than bobcats... but both mountain lions... diagnosed with anticoagulant intoxication died after spending the bulk of their last month in the most developed parts of their home ranges." Additionally, a mountain lion's diet is primarily composed of ungulates (primarily deer), although they will also eat rodents, insects, and smaller predators, depending upon location, season, and abundance (Iriarte et al (1990) and Riley et al (2007)). However, Riley et al (2007) found that "coyotes made up 15% and 7% of the kills for the 2 lions that died of anticoagulant intoxication." This suggests that the mountain lions that died due to anticoagulant toxicity spent more time in the developed part of their home ranges and were consuming more coyotes than the mountain lions that died due to other causes.

Of the 41 bobcats found in five counties and analyzed between 1995 and 2010, approximately 85% tested positive for second generation rodenticides, 76% tested positive for brodifacoum, 63% tested for bromadiolone, and 26% tested positive for difethialone (most of the coyotes tested positive for more than one rodenticide). Between 1995 and 2003, Riley et al (2007) analyzed 35 bobcats in the Santa Monica Mountains (including in the SMMNRA) and Simi Hills of Los Angeles and Ventura Counties. Approximately, 94% tested positive for second generation rodenticides, 82% tested positive for brodifacoum, 71% tested for bromadiolone, and 29% tested positive for difethialone. All nineteen bobcats that died due to severe notoedric mange also tested positive for second generation anticoagulant rodenticides, with brodifacoum ranging from trace to 0.56 ppm. In bobcats with levels of more than 0.05 ppm, the association to mange (and mortality) was “highly significant,” with a p-value < 0.01 (using a Mann-Whitney U test or a Fisher’s Exact test). Bobcats are considered strict carnivores and primarily consume rodents and rabbits, although they will also consume insects, reptile, and larger prey (including deer), depending upon availability, season, and preference (Litvaitis (1981)).

Of the 44 coyotes found in seven counties and analyzed between 1998 and 2010, approximately 75% tested positive for second generation rodenticides, 75% tested positive for brodifacoum, 27% tested for bromadiolone, and 9% tested positive for difethialone. Coyotes found in the SMMNRA (a natural area), in “urban” areas of Los Angeles and Ventura Counties, and unknown areas of Los Angeles and Ventura Counties between 1997 and 2003 were analyzed for rodenticides (Riley, 2012). Out of 25 coyotes, 76% tested positive for brodifacoum, 32% tested positive for bromadiolone, and 16% tested positive for difethialone. There was no statistically significant difference (using Chi-square) between the urban and the natural areas. Tables 12 and 13 summarize the results.

Table 12. Number of coyotes analyzed that had anticoagulant rodenticide residues (including trace residues) by location (land use) within Los Angeles and Ventura Counties from 1997 to 2003 (n=25)<sup>1,2</sup>.

Land type/ Population Density	Number of Coyotes	Second Generation Anticoagulant Rodenticide			First Generation Anticoagulant Rodenticide	
		Brodifacoum	Bromadiolone	Difethialone	Chlorophacinone	Diphacinone
Urban	14	11 (78.6%)	6 (42.9%)	4 (28.6%)	1 (7.1%)	2 (14.3%)
Unknown	5	4 (80%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)
SMMNRA (Natural Area)	6	4 (66.7%)	1 (16.7%)	0 (0%)	0 (0%)	2 (33.3%)
Total	25	19 (76%)	8 (32%)	4 (16%)	1 (4%)	4 (16%)

1. Samples may be positive for more than one rodenticide.
2. None of the samples were positive for warfarin.

DPR analyzed the coyotes from Los Angeles and Ventura Counties, Lima and Salmon’s raptor study, and the San Joaquin kit fox study, as a group so that the results could be compared. DPR

analyzed the animals by location (using land use and/or population density) for rodenticides. See Table 13, below.

Table 13. Number of animal analyzed that had anticoagulant rodenticide residues (including trace residues) by land use and/or population density from 1997 to 2011 (n=209)<sup>1,2</sup>.

Land type/Population Density	Number	Second Generation Anticoagulant Rodenticides			First Generation Anticoagulant Rodenticides	
		Brodifacoum	Bromadiolone	Difethialone	Chlorophacinone	Diphacinone
Urban <sup>5</sup>	116	96 (82.8%) <sup>4a</sup>	46 (39.7%) <sup>4a</sup>	7 (6.0%) <sup>4a</sup>	8 (6.9%)	4 (3.4%)
Unknown <sup>6</sup>	16	11 (6.9%)	9 (56.3%)	2 (12.5%)	1 (6.3%)	0 (0%)
Other <sup>7</sup>	20	6 (30.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Rural <sup>8</sup>	35	33 (94.3%) <sup>4a</sup>	13 (37.1%) <sup>4a</sup>	5 (14.3%) <sup>4a+</sup>	0 (0%)	0 (0%)
Natural <sup>9</sup>	23	6 (26.1%) <sup>4b</sup>	2 (8.6%) <sup>4b+</sup>	0 (0%) <sup>4b+</sup>	1 (4.3%)	3 (13.0%)
Total of these animals <sup>3</sup>	248	152 (61.3%)	70 (28.2%)	14 (5.6%)	10 (4.0%)	7 (2.8%)
Average of the evaluated data <sup>3</sup>	492	339 (68.9%)	183 (37.2%)	41 (8.3%)	18 (3.7%)	53 (10.8%)

1. Animals may be positive for more than one rodenticide. No animal was positive for warfarin.
2. Using a Chi-square test (with a Yates correction for continuity when appropriate (i.e., at least 20% of the cells had a frequency of less than 5 (per (a) Preacher (2001), (b) calculation, and/or (c) both))), the three second generation anticoagulant rodenticides (as a group, using the actual numbers in the table (i.e., not the percentages)) were analyzed at each land use type/the animal's location. When the notations are the same (eg, 2a and 2a) the locations did not differ statically significantly (p>0.05) from each other (Preacher (2001)). When they differ (eg, 2a and 2b), they are statically significantly different (p<0.05) from each other.
3. "Average data" is the cumulative data (from Tables 2 and 7) and is there for comparison to the total data.
4. Using a Chi-square test (with a Yates correction for continuity when appropriate, Preacher (2001)), the three second generation anticoagulant rodenticides (individually, using the actual numbers in the table (i.e., not the percentages)). When the notations are the same (4a-4b), the locations did not differ statically significantly (p<0.05) from each other. When they differ, they are statically significantly different (p<0.05) from each other. + indicates that because the number was so low, Yates was may have been used and/or it might have been inappropriate to utilize Chi-square.
5. The urban animals include: 1 badger, 1 skunk, 2 Cooper's Hawks, 2 Red-tailed Hawks, 2 Sharp-shinned Hawks, 4 Red-shouldered Hawks, 4 red foxes, 7 Barn Owls, 24 coyotes, and 69 San Joaquin kit foxes.
6. The animals from Unknown areas include: 1 Great Horned Owl, 5 coyotes, and 10 San Joaquin kit foxes.
7. The animals from Other areas include: 1 bobcat, 1 skunk, and 18 San Joaquin kit fox. Other Locations were designated by the study authors and include areas in Kern, San Benito, San Luis Obispo, and Tulare counties and could include urban, suburban, rural, agricultural, and/or natural areas.
8. The animals from Rural areas include: 1 American kestrel, 1 hawk, 2 Great Horned owls, 2 Sharp-shinned hawks, 4 Red-tailed hawks, 7 Red-shouldered hawks, 8 Cooper's hawks, and 10 Barn owls.
9. The animals from Natural areas include: 1 black bear, 9 coyotes, and 13 San Joaquin kit fox.

Even though Table 13 only utilizes subset of the data, and does not include most of the bobcats, mountain lions, foxes, coyotes, or the raptors from the CDFG data, it does include the bobcats and mountain lions from Riley et al (2007), the raptors from Lima and Salmon (2010 and 2012), the coyotes from Riley (2012), and the San Joaquin kit fox study from CDFG (2011 and 2012b). The data also show a statistical difference between the percent of animals with brodifacoum and bromadiolone in the rural and urban environments compared to the natural environment. However, it shows that there is no significant difference in the occurrence of difethialone in rural and urban even though the rodenticide occurred less frequently in natural areas.

### **Rodenticide Sales/Use Rates**

Two DPR databases were used to determine rodenticide use rates in California: Pesticide Use Report (PUR) and “Report of Pesticide Mill Assessments in California” (also referred to as the Mill Assessment Database). All agricultural pesticide use must be reported monthly to County Agricultural Commissioners, who in turn, report the data to DPR. The PUR is a yearly compilation of this data, (reported in total pounds of active ingredient (a.i.)). In California, the term “agricultural use” includes pesticide applications to crops, parks, golf courses, pastures, landscape maintenance, and roadsides/right of ways. Although not considered “agricultural use,” all applications made by licensed applicators, including structural application, public health application, and home and garden applications, are included in the PUR database. The PUR does not include applications of pesticides by homeowners or other non-licensed persons, including home and garden use, most industrial uses, and most institutional uses. The Mill Assessment Database indicates pesticide sales (in dollars) and quantity (in pounds or gallons) of all registered pesticides sold in California.

Table 14 compares the average total pounds of first and second generation anticoagulant rodenticide active ingredients sold per year between 2006 and 2010 in California, to the average total pounds of reported use of the same active ingredients for the same years. DPR then subtracted the average annual pounds sold by the average annual pounds reported used to estimate the average annual pounds of rodenticides used by non-licensed persons. For purposes of this analysis, DPR assumed a zero percent error between sales and unlicensed use of anticoagulant rodenticides. However, sales and use are not directly related to each other as a person may buy rodenticide one year, but not necessarily use the rodenticide that year or at all.

Table 14. A comparison of the average per year (2006 to 2010) of rodenticides sold (in pounds a.i.) to the average per year (2006 to 2010) of pounds of rodenticides reported used (PUR) (in pounds a.i.) to an estimated pounds of use of rodenticides by non-licensed personnel (calculated by subtracting the PUR from the total sold).

Type of Rodenticide	Rodenticide	Total Sold <sup>1</sup> (lbs. of a.i. (%))	PUR <sup>2</sup> (lbs. of a.i. (%))	Estimated Non-licensed Use <sup>3</sup> (lbs. of a.i. (%))
Second Generation Anticoagulant Rodenticides	Brodifacoum	26.58 (6.54%)	3.07 (2.66%)	23.51 (8.09%)
	Bromadiolone	51.02 (12.56%)	32.48 (28.10%)	18.54 (6.38%)
	Difencoum <sup>4</sup>	0.25 (0.06%)	0.015 (0.01%)	0.235 (0.08%)
	Difethialone	4.49 (1.1%)	3.64 (3.15%)	0.85 (0.29%)
First Generation Anticoagulant Rodenticides	Chlorophacinone	66.54 (16.38%)	17.42 (15.07%)	49.12 (16.79%)
	Diphacinone	226.99 (55.9%)	56.70 (49.05%)	170.29 (58.57%)
	Warfarin	30.44 (7.49%)	2.27 (1.96%)	28.17 (9.69%)
Total Rodenticides		406.32 (100.00%)	115.595 (100.00%)	270.485 (100.00%)

1. From the Mill Assessment Database.
2. From the PUR database. The PUR includes pesticide applications on parks, golf courses, pastures, structural pest control, landscape maintenance, roadsides/right of ways, and crops, and all pesticide applications made by licensed applicators.
3. Calculated by subtracting the “PUR” Use from the Total Sold. Estimates the rodenticides applied by non-licensed applicators (i.e., homeowners, building and maintenance workers, custodians).
4. Two (2) year (2009 and 2010) average.

If the pounds of anticoagulant rodenticides sold or reported used in California per year seem low, please note that the figures are in pounds of “active ingredient,” not pounds of product containing the active ingredient. Most anticoagulant rodenticides contain around 0.002% to 0.005% active ingredient. Therefore, over 200,000 pounds of formulated product containing the active ingredient brodifacoum were sold or used in California per year.

When reporting pesticide use to DPR, applicators must indicate a “use site.” Table 15 demonstrates how much (both in pounds of a.i. and percent) of the reported use of each anticoagulant rodenticide, between 2006 and 2010, was identified as used on a “Public Health,” “Regulatory Pest Control,” “Structural Pest Control,” or “Vertebrate Pest Control” use site.

Table 15. Reported annual use for Public Health, Regulatory Pest Control, Structural Pest Control and Vertebrate Pest Control separated out in pounds of active ingredient (lb of a.i.) and percentage that each use represents of the a.i. for each rodenticide of the seven anticoagulant rodenticides between 2006 and 2010.

Type of Rodenticide	Rodenticide	Total PUR <sup>1</sup> (lbs. of a.i.)	Public health (lbs. of a.i.) (% of use)	Regulatory pest control (lbs. of a.i.) (% of use)	Structural pest control (lbs. of a.i.) (% of use)	Vertebrate pest control (lbs. of a.i.) (% of use)	Other Uses (lbs. of a.i.) (% of use)
Second Generation Anticoagulant Rodenticides	Brodifacoum	3.07	0.004 (0.12%)	0.01 (0.32%)	2.62 (85.45%)	0.10 (3.10%)	0.336 (10.94%)
	Bromadiolone	32.48	0.61 (1.86%)	0.003 (0.01%)	28.11 (86.54%)	0.48 (1.49%)	3.277 (10.09%)
	Difenacoum <sup>2</sup>	0.015	0 (0.00%)	0.001 (6.67%)	0.008 (53.33%)	0.001 (6.67%)	0.005 (33.33%)
	Difethialone <sup>3</sup>	3.64	0 (0.00%)	0 (0.00%)	2.08 (57.20%)	0.01 (0.36%)	1.55 (42.58%)
First Generation Anticoagulant Rodenticides	Chlorophacinone	17.42	0 (0.00%)	0 (0.00%)	1.50 (8.58%)	2.18 (12.54%)	13.74 (78.87%)
	Diphacinone	56.70	0.19 (0.34%)	2.53 (4.47%)	39.19 (69.12%)	10.38 (18.30%)	4.13 (7.28%)
	Warfarin	2.27	0.003 (0.12%)	0 (0.00%)	0.19 (8.50%)	1.70 (74.67%)	0.377 (16.61%)

1. From the PUR database. The PUR includes pesticide applications on parks, golf courses, pastures, structural pest control, landscape maintenance, roadsides/right of ways, and crops and pesticide applications made by licensed applicators.
2. Two (2) year (2009 and 2010) average.
3. In 2010, the PUR for difethialone was likely reported in gallons instead of pounds, so a 4-year average for the Structural Use data was utilized (2006 to 2009).

Between 2006 and 2010, of the four second generation rodenticides, bromadiolone was the highest in terms of average annual total of pounds of active ingredient sold and reported used. Approximately 51 pounds of bromadiolone were reported sold, and approximately 33 pounds were reported used. Of the 33 pounds of bromadiolone reported used, approximately 87% was for structural pest control. DPR estimates that 19 pounds of bromadiolone were used by non-licensed persons.

Brodifacoum was the second highest second generation anticoagulant rodenticide in terms of average annual pounds of active ingredient sold. However, it is third highest in terms of pounds reported used. An average of approximately 27 pounds of brodifacoum active ingredient was sold annually in California over the four years. However, only three pounds of brodifacoum were reported used. Based on the difference between sales and reported use, DPR estimates that 89% of brodifacoum use was by non-licensed persons (homeowners, building and maintenance workers, custodians, etc.).

This information is not surprising as the majority of products containing brodifacoum were marketed for use by homeowners and non-licensed personnel, whereas the structural pest control industry has favored the use of bromadiolone. As shown in Table 14, both chemicals have been used in structural pest control, just by different types of applicators (i.e., licensed vs. unlicensed).

As shown in Table 15, there have been relatively few sales and/or reported use in California of either difethialone or difenacoum. This may be a reflection of the fact that these are the most recent second generation anticoagulant rodenticides to receive registration in California, not that these rodenticides will not cause a problem for non-target wildlife.

### **Uncertainties**

The scope of DPR's analysis is limited to available data. The data show that exposure and toxicity from second generation anticoagulant rodenticides is occurring to non-target wildlife. However, the data do not tie that exposure/toxicity to any particular rodenticide use pattern (e.g., indoor versus outdoor use of rodenticide). As mentioned above, DPR attempted to separate use of second generation anticoagulant rodenticides by licensed (professional) versus unlicensed personnel by subtracting the average pounds reported use from the average annual pounds sold. However, sales and use are not directly related to each other as a person may buy a rodenticide one year, but not necessarily use the rodenticide that year or at all. In addition, it is not known how much of the "estimated use" of second generation anticoagulant rodenticides by unlicensed persons is for industrial, institutional, home/garden, or other uses, and how much is correctly applied, accidentally mishandled, or intentionally misused.

Morzillo and Mertig 2011(a) found that only 10% of residents who used rodenticides were aware of the potential non-target effects. Additionally, Morzillo and Schwartz (2011) found that residents attempt to control target animals, as well as non-target pests and non-target carnivores, San Joaquin kit fox, coyotes, and bats, particularly in single-family homes. Bartos et al (2012) found that residents in the San Fernando Valley and Bel Air-Hollywood used rodenticides to target rats and mice, as well as opossums, snakes, and raccoons up to 300 feet from structures (the limit is 100 feet). Only 42% of participants admitted knowing that rodenticides might affect wildlife. PCOs were primarily called about outdoor landscaping and primarily used snap traps to control rats and mice. Of the 7 that responded, 4 used exclusion, 3 used second generation rodenticides, and 2 used first generation anticoagulants.

Additionally, there are known cases of illegal use. In 2010, the Forest Service cleaned up and restored 335 illegal marijuana sites in national forests in California, removing more than 300 pounds of pesticides (Ferrell (2011) and USDA Forest Service (2011)), including rodenticides which are used to protect the marijuana plants from rodents. Ferrell stated that, "anticoagulant rodenticide... contamination could contribute to continued decline of the Fisher's population." Additionally, according to Gurrola (2010), in certain counties, medical marijuana "has had problems with outdoor growers using massive quantities of rodenticides to protect their crops from rodents," which can cause "secondary poisoning to non-target species and... (m)edical marijuana patients."

## Summary

The data clearly indicate that exposure and toxicity to non-target wildlife from second generation anticoagulant rodenticides is a statewide problem. Research data from various locations throughout California indicate that exposure is occurring in many taxa and in every ecosystem. Mammals, birds, and even a reptile, have tested positive for second generation rodenticides. Based on the data provided, DPR believes that the exposure of wildlife to second generation rodenticides is a problem in both urban and rural areas. While the data show exposure and that these exposures put San Joaquin kit fox “in danger of extinction,” they do not link specific uses, or location of use of second generation anticoagulant rodenticide (i.e., indoors versus outdoors, homeowners versus professionals) that resulted in the exposure.

Additionally, although brodifacoum was found less often in the natural areas, second generation anticoagulant rodenticides were still found in animals in natural areas. The data also indicate that brodifacoum and difethialone are extremely toxic to both birds and mammals. Bromadiolone and difenacoum are moderately toxic to birds, but extremely toxic to mammals.

Brodifacoum was first registered for use in California in 1983. An average of 27 pounds of brodifacoum active ingredient were sold each year for the last five years, 12 pounds of which were reported used by licensed pest control applicators. While brodifacoum accounts for approximately 7% of all anticoagulant rodenticides sold, residues of brodifacoum were found in approximately 68% of the animals that DPR analyzed, including coyotes, bobcats, mountain lions, endangered San Joaquin kit foxes, and federally protected raptors. Of the animals analyzed between 1995 and 2011, brodifacoum was likely involved in approximately 13% of animal mortalities and was solely responsible for 9% of animal mortalities.

Bromadiolone was first registered in California in 1982. An average of 51 pounds per year of bromadiolone active ingredient was sold in California between 2006 and 2010, approximately 63% of which was reported used by licensed pest control applicators. Of the rodenticides sold in California, bromadiolone accounted for approximately 13% of anticoagulant rodenticide use. Bromadiolone residues were found in approximately 36% of the animals analyzed, including coyotes, bobcats, mountain lions, endangered San Joaquin kit foxes, and federally protected raptors. Between 1995 and 2011, bromadiolone was likely involved in approximately 3% of animal mortalities.

Difethialone was first registered for use in California in 1997. Difethialone accounts for approximately 1% of anticoagulant rodenticide sales, with approximately 80% reported used by licensed pest control applicators. Residues were found in approximately 8% of the animals analyzed, including bobcats, mountain lions, coyotes, and federally protected raptors. While DPR has no data indicating that difethialone was directly involved in an animal mortality, the data do indicate that the percent of animals with difethialone residues above the most sensitive LD<sub>50</sub> is relatively high compared to the percent of difethialone sold. Based on its half-life and toxicity data, difethialone appears to be most similar to brodifacoum.

Difenacoum, the newest second generation anticoagulant rodenticide, was first registered with the DPR in 2008. Between 2009 and 2010, difenacoum accounted for approximately 0.3% of the anticoagulant rodenticide that was sold, almost all of which was sold for unlicensed use. In England, between 1998 and 2006, there were eight to 36 “wildlife incidents” per year involving difenacoum. The affected animals included raptors, song birds (i.e., passerines), game birds, domestic animals (dogs and cats), wild canids, and rodents (U.S. EPA, 2007). Based on its half-life and toxicity data, difenacoum appears to be most similar to bromadiolone.

The data also show that exposure of wildlife to second generation anticoagulant rodenticides can lead to sub-lethal effects. Multiple studies have shown that sub-lethal doses can cause lethargy, shortness of breath, anorexia, bloody diarrhea, and tenderness of the joints. Riley et al’s (2007) study of bobcats is an example of sub-lethal effects. Mortality in bobcats due to notoedric mange had not previously been reported as a significant pathogen in wild felid; mange has been strongly correlated to brodifacoum ( $p < 0.05$ ), but has not been shown to be caused by rodenticides. This shows that even sub-lethal exposures to anticoagulants may contribute to the ill thrift of the animal and hence the mortality in a wild animal. In addition, to date, very few studies have looked at rodenticide residues in fetuses or in newly whelped or hatched animals. Klein Sereiy’s (2012) data, which found residues in a bobcat fetus, indicate that rodenticides are able to pass the placental barrier. The sub-lethal effects of rodenticides reduce the biological fitness of wildlife.

### **Conclusion**

Based on the data above, DPR finds that use of two of the four second generation anticoagulant rodenticides--brodifacoum and bromadiolone-- present a hazard related to persistent residues in target animals resulting in impacts to non-target wildlife. Because they are similar in half-life and toxicity, DPR also find that if the use of difethialone and difenacoum were to increase, rodenticides containing those two second generation anticoagulant rodenticides may also present a hazard related to persistent residues in target animals.

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## **APPENDIX I: Non-California Data**

DPR also evaluated studies conducted in locations other than California. When the data came from a rodent eradication effort, rodenticide use rates were significantly higher than normal label rates. However, the data are still useful for presenting the potential impacts of rodenticides.

Howald et al (2009) utilized brodifacoum to eradicate black rats (*Rattus rattus*) from the three islets of Anacapa Island. An endemic mouse (the Anacapa deer mouse) and several protected birds also inhabited the island. Even though the organizers employed several measures to reduce mortality of the non-target organisms, at least 94 birds were found dead after the bait application including 6 Burrowing Owls, an American Kestrel, 3 Barn Owls, and multiple thrushes.

The Department of Environmental Conservation (2012) necropsied and ran rodenticide analysis on 4 Red-tailed Hawks found dead in Manhattan, New York. One had residues of difethialone, 2 had residues of difethialone, brodifacoum, and bromadiolone, and 1 had residues of difethialone, brodifacoum, bromadiolone, and diphacinone. Based on necropsies conducted by veterinarians, 3 died due to anticoagulant rodenticide poisoning and 1 died due to “complications due to egg laying (oviductal prolapse), possibly exacerbated by hemorrhaging.” The Department of Environmental Conservation concluded that at least 1 of the Red-tailed Hawks most likely died directly from difethialone toxicity.

Stone et al (1999) documented 52 non-target wild animals that appeared to have died due to anticoagulant rodenticide toxicosis in New York between 1989 and 1997. Brodifacoum was found in over 90% of the animals. Raptors (primarily Great Horned Owls and Red-tailed Hawks) comprised half the cases. Eastern gray squirrels, raccoons, and white-tailed deer were the mammals that were most frequently poisoned.

Murray (2011) analyzed the livers of 4 raptor species presented to a wildlife rehabilitation clinic between April 2006 and March 2010. All either died or were euthanized. Of the 161 birds, 139 (86%) had residues of anticoagulant rodenticides, including 100% of the Great Horned Owls, 89% of the Red-tailed Hawks, 87% of the Eastern Screech Owls, and 75% of the Barred Owls. One-hundred thirty-six animals had residues of brodifacoum, including 99% of the positive birds. One Barred Owl and 1 Red-tailed Hawk were positive for both brodifacoum and difethialone, and 1 Barred Owl was positive for bromadiolone. Rodenticide toxicosis was identified as the cause of death in nine animals (5.6% of the animals), all of which had brodifacoum residues.

Howald (1997) examined the Canadian Wildlife Service’s attempt to eradicate the Norway rat (*Rattus norvegicus*) from Langara and Lucy Islands using brodifacoum in baiting stations. Of the radio-collared Norway rats between 13.4% and 33.3% died above ground and some appeared to have been scavenged. Thirteen (100%) ravens tested positive for brodifacoum (with a liver brodifacoum range of 0.985 to 2.522 ppm). The cause of death was confirmed at necropsy and

none of the birds were in poor body condition or had any evidence of other diseases. Crows tested positive for brodifacoum up to nine months after the baiting ceased and bald eagles were also confirmed to be exposed. Crows and ravens were observed eating rats and the bait. Snails, slugs, blowfly larva, and other species also tested positive for brodifacoum.

Several papers have suggested that invertebrates might be potential sources of rodenticides to animals that predate invertebrates (Booth et al (2001), Booth et al (2003), Brakes and Smith (2005), Craddock (2003), Fisher et al (2011), Ogilvie et al (1997), and Shlosberg and Booth (2001)). Weta, cockroaches, beetles (*Holcaspis stewartensis* and *Mecodema*), locuses, and land crabs all tested positive for brodifacoum (range: 0.02 to 7.47 ug/g), after either directly consuming or being gavaged with brodifacoum. While the animals themselves appeared relatively insensitive to brodifacoum (with no mortality reported), these animals can travel up to 10 meters and it could take more than ten weeks for the brodifacoum to return to pre-baiting levels. Additionally, brodifacoum caused mortality in three species of snails (*Pachnodus silhouettanus*, *Achatina fulica*, and *Pachystyla bicolor*).

Albert et al (2009) collected 164 dead owls (Barn, Barred, and Great Horned Owls) in Canada. Albert et al conducted necropsies and analyzed the livers for seven rodenticides (brodifacoum, bromadiolone, chlorophacinone, diphacinone, difethialone, pindone, and warfarin). Of the samples, 70% had detectable residues of at least one rodenticide. The prevalence of brodifacoum was approximately 50% and the prevalence of bromadiolone was approximately 52%. Nine of the birds (approximately 6%) were assigned anticoagulant rodenticide poisoning as the “final cause of death.”

Thomas et al (2011) analyzed data (from the previous 10 years, including from Albert et al (2009)) of 270 birds (including 196 Great Horned Owl and Red-tailed Hawks) from Canada using logistic regression to estimate the probability of rodenticide toxicosis at various levels of second generation anticoagulant rodenticides. They found that approximately 65% of the Great Horned Owls and Red-tailed Hawks had residues of at least one second generation anticoagulant rodenticide and that approximately 11% of Great Horned Owls were at risk of dying directly due to the effects of second generation anticoagulant rodenticides.

Lambert et al (2007) collected 58 dead birds (including raptors and water birds) from Loire Atlantique, France, conducted necropsies on them, and had their livers analyzed for five rodenticides, including brodifacoum, bromadiolone, and difenacoum. Bromadiolone residues were found in 26% of the animals and difenacoum residues were found in approximately 14% of the animals. Based on the results of the necropsies, none of the animals appeared to have died directly from anticoagulant rodenticide toxicity.

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**Summary**

The above data indicate that non-target animals from locations outside of California have also been impacted by second generation anticoagulant rodenticides.

## **Appendix II: Additional Information and Data in California**

Additional California data that did not fit easily into DPR's main analysis and/or needed additional explanation are summarized in this section:

### **Reptiles**

Measurable levels of difethialone were found in a gopher snake in the Los Angeles area (Klein Sereiys (2012)). Fisher and Saunders (2012) found that Galapagos tortoises were unlikely to consume brodifacoum. However, Hoare and Hare (2006) found that 2 species of New Zealand geckos would consume bait. Spurr (1993) reported a case where over 100 skinks (*Leiolopisma otagense* and *L. grande*) were found dead after an eradication effort using brodifacoum. Harper et al (2011) found a mortality rate of approximately 5% among Galapagos land iguanas after an eradication program for the black rat (*Rattus rattus*) using brodifacoum on Seymour Norte, Galapagos. Eason and Spurr (1995) concluded that reptiles and amphibians "may be at risk from secondary poisoning" especially if they consumed invertebrates that had fed on brodifacoum. This data indicates that reptiles may also be impacted by anticoagulant rodenticides.

### **Fetal and neonatal data**

The fetus of a bobcat that was hit by a car in the Los Angeles area contained residues of brodifacoum and diphacinone (Klein Sereiys (2012)). Additionally, 1 of 4 fisher kits (that were nursing as their sole source of nutrition) contained trace levels of brodifacoum (Gabriel et al (2012)). The bobcat and kit data suggest that neonatal and lactation transfer are two additional possible routes of exposure for anticoagulant rodenticides that may result in impacts to wildlife.

To date, very few studies have looked at rodenticide residues in fetuses or in newly whelped or hatched animals. In humans, anticoagulants are known to induce two different effects, depending on the time of exposure. Fetal warfarin syndrome is characterized by nasal hypoplasia, causing respiratory difficulty. Fetal wastage results in nervous system, skeletal, and ophthalmological abnormalities causing blindness, low birth weight, and developmental delays (Howald (1997)).

Munday and Thompson (2003) found that two newly whelped puppies that died shortly after birth had brodifacoum residues and showed signs of rodenticide toxicity. The puppies had signs of coagulopathy and were statistically significantly smaller than the unaffected puppies, even though the dam and five unaffected puppies were clinically normal. The authors stated that, "the dam was unaffected, suggesting that fetuses are more susceptible to brodifacoum toxicity than adult animals." If this is the case, then even healthy animals that have residues of anticoagulant rodenticides and are pregnant might lose their offspring due to the effects of the rodenticides.

Naim et al (2011) compared the breeding performance of Barn Owls in Oil Palms that were in an untreated control or had been treated with warfarin, brodifacoum, or a bio-rodenticide (*Sarcocystis singaporensis*, a parasitic protozoon) in three successive seasons. The researchers found no difference in the clutch size based on treatment. However, there was a statistically

significant difference in hatching success. Brodifacoum resulted in a hatching success rate of approximately 43%, whereas the control showed a hatching success rate of approximately 84%. Fledging success was also statistically different among the 4 treatments, with the control showing approximately 78% success and those exposed to brodifacoum showed 10% success. In all three seasons, brodifacoum was correlated to the lowest hatching and fledging success (statistically significant from all other treatments at  $p < 0.05$ ).

### **Difethialone in Hawks and a Fox in San Francisco**

In San Francisco's Golden Gate Park, 4 hawks and a fox appear to have been affected by "ingesting rats poisoned by difethialone that was used to control rodents in the park." Three of the hawks and the fox are believed to have died as a result of ingestion. The fourth hawk was sent to a wildlife rehabilitation center for treatment and was released (Kay (2007a) and Kay (2007b)). The laboratory and necropsy data were not available for analysis.

### **Summary**

These data indicate that reptiles and amphibians, and fetuses and newly born/ hatched animals may also be impacted by anticoagulant rodenticides. The data indicate that rodenticides are able to pass the placental barrier.

**Appendix III: Fishers and Badgers in California**

DPR recently received data from the analysis of 64 mustelids (fishers and badgers) in California.

**Analysis**

The data were collected between 2005 and 2011, and came from Gabriel et al (2012a) and Quinn et al (2012). The analysis includes data on 58 fishers and six badgers.

The livers of each animal were analyzed for seven anticoagulant rodenticides. The animals were analyzed for first generation anticoagulant rodenticides - chlorophacinone, coumachlor, diphacinone, and warfarin-- and second generation anticoagulant rodenticides -- brodifacoum, bromadiolone, and difethialone.

Of the 64 non-target animals analyzed, 75.0% had residues of at least one second generation anticoagulant rodenticide. Brodifacoum residues were found in approximately 73% of the animals, bromadiolone residues were found in approximately 30% of the animals, and difethialone residues were found in approximately 2% of the animals. Table 1 summarizes the results.

Table 1. Number (and percent) of the rodenticides among fishers and badgers (n=64) and among positive fishers and badgers (n=50)<sup>1</sup>.

Total	Number	Second Generation Anticoagulant Rodenticides			First Generation Anticoagulant Rodenticides		
Samples	64	48 (75.0%)			>8 (>12.5%)		
Positives	50	48 (96.0%)			>8 (>16.0%)		
Total	Number	Brodifacoum	Bromadiolone	Difethialone	Chlorophacinone	Diphacinone	Warfarin
Samples	64	47 (73.4%)	19 (29.7%)	1 (1.6%)	4 (6.3%)	8 (12.5%)	1 (1.6%)
Positives	50	47 (94.0%)	19 (38.0%)	1 (2.0%)	4 (8.0%)	8 (16.0%)	1 (2.0%)

1. Animals may be positive for more than one rodenticide.

Necropsies

Out of the 64 animals analyzed for rodenticides, 58 had necropsies conducted at the California Animal Health and Food Safety Laboratory System (CAHFS) or the Veterinary Medical Teaching Hospital (VMTH), both part of the University of California at Davis located in Davis, California.

A summary of 4 (6.9%) fisher necropsies, where the fishers were most likely killed by anticoagulant rodenticides between 2009 and 2011, were included in the article (Gabriel et al (2012a)). The 4 animals included 2 from the Sierra Nevada population and 2 from the Northern population. Of the 4 animals that most likely died due to anticoagulant rodenticide toxicity, 1 had residues of brodifacoum and chlorophacinone and 3 had residues of bromadiolone and

brodifacoum. All 4 had detectable levels of brodifacoum, with the levels ranging from 0.04 to 0.61 ppm.

### **Location & Land Use**

The fishers and badgers came from ten different California counties, including Fresno, Humboldt, Los Angeles, Madera, Mariposa, Monterey, Shasta, Siskiyou, Tehama, and Trinity. The data indicate that fishers and badgers found in rural/agricultural, as well as those found in nature preserves/National Forests, were positive for anticoagulant residues.

Fishers are a candidate for listing under the federal Endangered Species Act in California. They are a medium-sized mammal in the mustelid (weasel) family. They are omnivores, consuming a wide variety of prey (such as rabbits, mice, squirrels, reptiles, amphibians, insects, porcupines, and carrion), as well as fruit, berries, and plants. In California, fishers “are dependent on mid to late-serial stage coniferous and hardwood forests” and often inhabit lands associated with a lack of humans. Gabriel et al (2012) used spacial analysis and found that exposure was widespread and not isolated to areas of known human activity. They came to the conclusion that a “likely source of AR exposure to fishers is... illegal marijuana cultivation.” This was supported by spacial analysis, the timing of the mortalities, and raids in areas surrounding the mortalities (Gabriel et al (2012a), Gurrola (2010), and USDA Forest Service (2011)).

The population of the fishers in the Sierra Nevada is estimated to be 150 to 300 individuals. There is no natural movement to or from the Sierra Nevada population to other populations (including the Northern California population), so individuals are gained through birth and lost through death. Forty (40) animals were analyzed from the southern Sierra Nevada population. Of these, 33 (82.5%) of the fishers were exposed to anticoagulant rodenticides, 32 (80.0%) were exposed to brodifacoum, 14 (35%) were exposed to bromadiolone, and 1 was exposed to difethialone. Two (5%) of the fishers died due to second generation anticoagulant rodenticide toxicity.

American badgers are primarily carnivorous, preferring to eat small burrowing mammals such as moles, ground squirrels, rats, mice, and gophers. They live in open areas (i.e., not forests or urban areas), including grasslands, parks, and farms. Data indicate that 4 of the 6 badgers were positive for second generation anticoagulant rodenticides, including 3 (50.0%) which were positive for brodifacoum, 3 (50.0%) which were positive for bromadiolone and 2 (33.3%) which were positive for both brodifacoum and bromadiolone.

### **Summary**

These data further support DPR’s assertion that exposure and toxicity to non-target wildlife from second generation anticoagulant rodenticides is a statewide problem, and that the use of second generation rodenticides presents a hazard related to persistent residues in target animals resulting in impacts to non-target wildlife.