Human Health and Ecological Risk Assessment for the Use of Wildlife Damage Management Methods by APHIS-Wildlife Services

Chapter XXX

USE OF DIPHACINONE IN WILDLIFE DAMAGE MANAGEMENT

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EXECUTIVE SUMMARY

Diphacinone is a rodenticide used by APHIS Wildlife Services (WS) to control various rodents and invasive mongooses in terrestrial environments. The registered uses of diphacinone include control of rodents and mongooses at agricultural sites (orchards, cropland, rangeland, and forest), in and around animal burrows and buildings (industrial, commercial, agricultural, and public), as well as non-crop areas (such as sewers, dumps, irrigation ditches, along fences, and gullies), and for conservation purposes (e.g., islands, grounded vessels). WS uses diphacinone baits (98 pound annual average from FY11-FY20) to reduce damage from pocket gophers, rats, mice, and mongooses.

As a first-generation anticoagulant rodenticide (FGAR), diphacinone usually requires multiple feedings by rodents to deliver a lethal dose. FGARs inhibit the production of active vitamin K, which decreases the synthesis of blood coagulation factors. This disturbance of clotting factors in the blood interferes with blood coagulation and leads to bleeding at capillaries, widespread internal hemorrhage, and death from excessive bleeding generally within 5 to 7 days.

APHIS evaluated the human health and ecological risk of diphacinone under WS proposed use patterns. The risk to human health, including workers applying diphacinone baits, is expected to be low based on the use pattern. The low risk to workers was determined based on label requirements regarding use of appropriate personnel protective equipment designed to reduce exposure. Risk to the public is not expected because WS uses tamper-resistant bait stations in areas accessible to the public, reducing potential exposure to the public, including children. Current broadcast applications for conservation use occur primarily on uninhabited islands or remote portions of inhabited islands. In cases where the public has access to applications on islands, tamper-proof bait stations may be used, as well as signage to reduce risk.

Diphacinone risk to terrestrial plants and aquatic environments is negligible based on the WS use pattern. WS has no records of nontarget species taken through its use of diphacinone bait. However, APHIS recognizes it is not possible to catalog all nontarget take because exposed animals may die belowground, be difficult to locate in certain habitats, or are wide-ranging and dispersed outside the treatment area. WS may monitor treatment sites to collect carcasses found aboveground to minimize secondary exposure to nontarget animals. WS avoids treating areas with threatened or endangered species that could be affected (many conservation treatments are to protect or are in close proximity to sensitive species) as well as burrows where nontarget species are detected. Despite this monitoring, APHIS recognizes nontarget species can be exposed to diphacinone bait. APHIS expects most of the target animals it baits belowground with diphacinone will die underground, reducing exposure potential to nontarget species; however, some will die aboveground.

The product labels instruct applicators to collect and dispose of dead or exposed animals during and after treatment, and some labels instruct to remove leftover bait but do not provide the time interval after treatment for doing this. Despite these mitigations, poisoned animals that are aboveground between searches or are missed during searches remain available to predators and scavengers. Birds are not as sensitive to diphacinone as mammals, and the risk of mortality to birds feeding on bait or animals with diphacinone residues is low. However, birds may experience sublethal effects. APHIS finds its use of diphacinone will have the greatest risk to mammals that access treated burrows or are in an area that receives an aboveground broadcast treatment where they may ingest bait or animals with diphacinone residues.

1 INTRODUCTION

The United States Department of Agriculture (USDA) Animal Plant Health Protection Service (APHIS) Wildlife Services Program (WS) controls populations of several rodent species and mongooses with the first-generation anticoagulant rodenticide (FGAR) diphacinone. Anticoagulants interfere with blood clotting, lead to bleeding at capillaries and internal bleeding, and cause death from excessive bleeding, generally within 5 to 7 days. Specifically, anticoagulants inhibit the formulation of prothrombin, which is a critical component in blood clotting (USEPA 2020b). As a first-generation anticoagulant, diphacinone usually requires multiple feedings by the target animal to deliver a lethal dose.

Diphacinone is a rodenticide first registered as a vertebrate control agent in the U.S. in 1962(USEPA 2015a). The registered uses of diphacinone include rodent control at agricultural sites (orchards, cropland, rangeland, and forest), in and around buildings (industrial, commercial, agricultural, and public), non-crop areas (such as sewers, dumps, irrigation ditches, along fences, and gullies, landscaping, and lawns), and for conservation purposes in rodent eradication efforts on islands (USEPA 2015a).

WS controls populations of several rodent species, including black rats[1], brown rats, Pacific rats, house mice, meadow voles, woodrats, cotton rats, pocket gophers, and small Indian mongooses (*Urva auropunctata*)[2] with diphacinone to prevent or minimize damage to property, crops, trees, shrubs, levees, and vulnerable native species and ecosystems. WS manages rodent populations and the damage they cause per label instructions by placing diphacinone baits where these species feed. However, WS very rarely conducts commensal rodent control in urban areas.

This human health risk assessment and ecological risk assessment evaluates potential risks and hazards to human health, nontarget fish, and wildlife from exposure to diphacinone from WS proposed use. The methods used for the human health risk assessment to assess potential human health effects follow standard regulatory guidance and methodologies and generally conform to other Federal agencies such as U.S. Environmental Protection Agency (USEPA) (National Research Council 1983, USEPA 2022). The methods used for the ecological risk assessment to assess potential ecological risk to nontarget fish and wildlife generally follow USEPA methodologies (USEPA 2022).

This risk assessment uses a standard approach of first identifying the hazard during problem formulation. Next, the toxicity of the hazard is evaluated in the dose-response assessment, followed by the determination of potential exposure populations and pathways. Lastly, the toxicity and exposure assessment information are integrated into the risk characterization (determining whether there is adverse human health and ecological risk). This risk assessment also includes a discussion of the uncertainties associated with the risk assessment and cumulative effects.

1.1 Use Pattern of Diphacinone

Diphacinone is commercially available in multiple pesticide products registered with the USEPA under the Federal Insecticide, Fungicide, and Rodenticide Act; formulations contain 0.005% diphacinone. Two of the diphacinone products used by WS are classified as restricted use pesticides (RUP), which may only be applied by certified pesticide applicators. The others used

[1] Scientific names are given in Chapter I: Introduction to the Risk Assessments.

[2] The small Indian mongoose has had changes in its common (small Asian mongoose) and scientific (*Herpestes javanicus*) names since the Chapter I: Introduction was written.

by WS are general use and can be used by WS and the public. Diphacinone comes in prepared bait blocks, pelletized and granular (e.g., hulled oat groats) baits, or tracking powder formulations.

From FY11[3]–FY20, WS used four diphacinone products under three Section 3 labels and one Special Local Needs (SLN) label under an additional Section 3 parent label. WS proposes to continue to use these formulations in wildlife damage management (Table 1). These formulations are pellets or bait blocks. It should be noted that WS used a fifth label in FY21, Apple Bait Block® Rodenticide (EPA Reg. No. 56-74), that was included in Table 1 (this label has the same use restrictions as Bait Block® Rodenticide with Peanut Butter Flavorizer™; EPA Reg. No. 56-42). Each diphacinone formulation has specific label instructions that applicators must follow when applying the baits (Table 1). Depending on the formulation's label, diphacinone products are applied via aircraft, bait boxes, cloth bags, hand bulb dusters, hand probes, spoons, spreaders, and bait stations (USEPA 2015a).

WS applicators primarily apply diphacinone pellet and block baits using tamper-resistant bait boxes/stations (commensal rodents), hand spot baiting directly into subterranean burrow systems of specific burrowing rodents (e.g., pocket gophers) following label instructions to ensure that people and pets cannot access the baits. Mechanical broadcast bait spreaders, aerial applications, or spot treatments could occur on islands for invasive commensal rodents (under the Dipacinone-50 Conservation label; EPA Reg. No. 56228-35). Application rates vary with the density of rodent populations, the target species being controlled, and the potential nontarget species present.

Hand spot baiting involves placing the bait within an active burrow opening or scattering it on trails, runways, or bare ground adjacent to burrow entrances and covering each placement with grass or shingle to avoid exposing nontarget organisms (restricted to Diphacinone-50 Conservation).

For open burrow systems, applicators may directly insert bait into burrows. For pocket gophers, specifically, a probe is often used to find the burrow; applicators find the burrow by pressing a probe into the ground about a foot from gopher mounds to find the main runway (the probe will easily drop when it hits a burrow). A hole is opened, and a four-ounce block is placed as far into the runway as the applicator can reach (USEPA 2019b). The hole created by the probe is then covered with debris and dirt while being careful not to cover the bait with dirt. Covering the hole discourages pocket gophers from plugging it and covering the bait with dirt. The number of applications per pocket gopher burrow system often depends on the number of burrow mounds because baits are placed a foot to a yard away from each mound in the main underground runways.

The labeled uses for Diphacinone-50 Conservation are for the control or eradication of brown, black, and Pacific rats, house mice, and other types of invasive rodents on islands for conservation purposes or on grounded vessels or vessels in peril of grounding (USEPA 2019a). The Diphacinone-50 Conservation label allows manual applications with tamper-resistant bait stations, burrow baiting, bait bolas (sachets), or ground and aerial broadcast methods (USEPA 2015a;2019a). The rate of application and frequency of treatment depends on the label-specified application method and target species. Bait stations must be tamper-resistant and prevent access by children, pets, and nontarget wildlife larger in body size than the target species (USEPA 2019a).

[3] FY11 equals the federal Fiscal Year 2011 which is October 1, 2010–September 30, 2011 (the year is denoted by FY11, FY12, and so on and is the federal Fiscal Year for 2011, 2012, and so on.

Table 1. Diphacinone products WS used in its animal damage management program (FY11-20) and proposes to use in the future.

Product Brand Name (registrant)	USEPA Registration Number (label date)	Formulation (0.005% Diphacinone)	Application Rate	Labeled Target Animals
Diphacinone-50 Conservation (Alternate Brand Names: *Diphacinone-50 [Conservation]: Pelleted Rodenticide Bait for Conservation Purposes*)	56228-35 (11/12/2019) Restricted Use Pesticide	Pellets	Several rates are provided on the label and are adjusted based on the application method, location, and target species	Norway [brown] rats, roof [black] rats, Polynesian [Pacific] rats, house mice, other types of invasive rodents on islands for conservation purposes, or on grounded vessels or vessels in peril of grounding
Ramik® Mini Bars All-Weather Bait	61282-26* (9/23/2021)	Bait blocks	Rates are variable depending on the target species. Can only be used within 100 feet of structures in bait stations (no burrow baiting)	House mouse, Norway [brown] rat, roof [black] rat, cotton rats (*Sigmodon hispidus*), eastern harvest mouse (*Reithrodontomys humuli*), golden mouse (*Ochrotomys nuttalli*), Polynesian [Pacific] rat, meadow vole, white-throated woodrat (*Neotoma albigula*), southern plains woodrat (*N. micropus*), Mexican woodrat, bushy-tailed woodrat
Ramik® Mini Bars All-Weather Bait	SLN No. HI-980005 (expires 8/13/2024) Restricted Use Pesticide	Bait blocks	Bait stations with 16 oz. of bait (number stations and distance variable)	Small Indian mongoose, house mouse, brown rat, black rat, Pacific rat, and other invasive rodents in Hawaii for conservation purposes
J.T. Eaton™ Answer® for the Control of Pocket Gophers (referred to as Eaton's Gopher Bait Block in this risk assessment)	56-57 (9/5/2019)	Bait blocks	Manual belowground applications. One 4-oz. bait block into each opened hole into runway, 2–3 locations in each main runway system	Botta's [Valley] pocket gophers, northern pocket gophers, Mazama [western] pocket gophers, Townsend's pocket gophers (*Thomomys townsendii*), Sierra or mountain pocket gophers (*T. monticola*), and plains pocket gophers in rangeland, cropland, forests, and noncrop areas
Bait Block® Rodenticide with Peanut Butter Flavorizer™	56-42 (9/23/2015)	Bait blocks	Rats: 4 to 16 1-oz blocks per placement at 15-30 ft. intervals Mice: 1 to 2 1-oz blocks per placement at 8–10-ft intervals Use in tamper-resistant bait boxes aboveground	Norway [brown] rats, roof [black] rats and house mice in and within 100 feet of man-made structures constructed in a manner so as to be vulnerable to commensal rodent invasions and/or to harboring or attracting rodent infestations
Apple Bait Block® Rodenticide (used minimally in FY21)	56-74 (9/23/2015)	Bait blocks	Rats: 4 to 16 1-oz blocks per placement at 15-30 ft. intervals Mice: 1 to 2 1-oz blocks per placement at 8–10-ft intervals Use in tamper-resistant bait boxes aboveground	Norway [brown] rats, roof [black] rats and house mice in and within 100 feet of man-made structures constructed in a manner so as to be vulnerable to commensal rodent invasions and/or to harboring or attracting rodent infestations

*WS did not use this bait from FY11–FY20 but it is the parent label for the SLN bait

Bait stations are allowed for all use sites specified on the label, all of which are non-crop areas. Burrow baiting is only permitted in uninhabited non-crop areas, and bait bolas are allowed for use on uninhabited grounded vessels or vessels in peril of grounding. Bait bolas may also be used in tree and shrub canopies in non-crop areas. Aerial and ground broadcast applications are not permitted on vessels or in areas of human habitation. Aerial broadcast applications are also not permitted when sustained winds exceed 35 miles per hour (mph) (USEPA 2019a). Warning signs must be posted in treatment areas that are accessible to the public (USEPA 2019a). The label instructs applicators to periodically monitor the baited area and remove dead carcasses during and after treatment operations. WS makes diphacinone applications under the Diphacinone-50 Conservation label for invasive rodent eradication or control projects on select islands to protect the islands' native animals and plants.

WS uses Ramik® Mini Bars All-Weather Bait (EPA Reg. No. 61282-26) under a Special Local Needs (SLN) label to control house mice, Pacific rats, and black rats in Hawaii. WS may also use this SLN product to control small Indian mongooses in Hawaii and, if labeled, in the Caribbean Islands. The parent label and Hawaii SLN label require the use of tamper-resistant bait stations for all outdoor aboveground applications and when applications are made in areas accessible to children, pets, and nontarget mammals and birds (USEPA 2019c;2021b). The parent label allows use in and within 100 feet of man-made structures, including homes, food processing facilities, industrial and commercial buildings, trash receptacles, agricultural and public buildings, transport vehicles (ships, trains, aircraft), docks, and port or terminal buildings. The parent label does not allow fence and perimeter baiting beyond 100 feet from a structure, direct application to food or feed crops, or burrow baiting. The parent label restricts use directly to water, areas where surface water is present, or intertidal areas below the mean high-water mark. The Hawaii SLN label specifies the use of tamper-resistant bait boxes with bait secured on rods within the bait stations preventing bait removal "in native ecosystems, such as forests, wetlands, coastal areas, and offshore islands, and other non-crop areas to protect native Hawaiian plants and animals" (USEPA 2019c). The Hawaii SLN label requires bait stations to be anchored to the ground or trees to prevent access to bait in areas prone to vandalism or where feral pigs are present.

WS also uses J.T. Eaton™ Answer® for the Control of Pocket Gophers (EPA Reg. No. 56-57; referred to as Eaton's Gopher Bait Block in this risk assessment) to control pocket gophers, primarily in New Mexico. The product provides a homeowner use label in addition to a professional use label. WS applies the professional use label for the control of pocket gophers in rangeland, orchards, vineyards, cropland, forest, and non-crop areas, including golf courses, parks, nurseries, and around homes (USEPA 2019b). Applicators place the bait blocks into burrows underground, using two to three bait blocks per burrow (USEPA 2019b). The label does not allow for aboveground use. The label does not allow direct application to the water where surface water is present or to intertidal areas below the mean high-water mark. The label requires checking the treatment area 10–15 days after treatment to remove any visible dead or dying pocket gophers.

WS lastly may use Bait Block® Rodenticide with Peanut Butter Flavorizer™ (EPA Reg. No. 56-42) and the similar Apple Bait Block® Rodenticide (EPA Reg. No. 56-74) to control brown rats, black rats, and house mice in and within 100 feet of man-made structures constructed in a manner to be vulnerable to commensal rodent invasions and/or to harboring or attracting rodent infestations (USEPA 2015b) on islands with these structures to protect bird nesting sites. The label defines structures to include homes, food processing facilities, industrial and commercial buildings, trash receptacles, agricultural and public buildings, transport vehicles (ships, trains, aircraft), and docks and port of terminal buildings. Applications per the label are placed by hand in areas rodents occur or placed in tamper-resistant bait stations. The label instructs the applicator to provide an uninterrupted supply of fresh bait for at least 10 days for rats, 15 days for mice, or until animal

activity ceases. The label does not allow use in food or feed crops. Broadcast bait and burrow baiting are prohibited. The label does not allow applications to water, areas where surface water is present, or intertidal areas below the mean high-water mark. The label requires collecting and disposing of all dead animals and leftover bait.

In the Management Information System (MIS)[4], WS personnel generally record the number of acres or burrows treated or estimate the number of target species taken with treatments. The quantity of bait applied is always recorded in the MIS. Most burrowing rodents taken with rodenticides die belowground (Nolte and Wagner 2001, Lee and Hyngstrom 2007, Ramey et al. 2007). Thus, counting the exact number of burrowing rodents taken with treatments is not possible because WS does not dig up burrows to determine the actual take of target or nontarget species.

If WS specialists estimated take in the MIS, that number was used for the average annual take numbers. If the take was not estimated in the MIS, take numbers were estimated based on the average occupancy of burrows by adults and young (number per burrow) or were estimated based on the expected number of target animals taken per pound of bait applied (number per pound) or expected density (number per acre). In general, these methods likely overestimate actual take numbers because it assumes 100% exposure and take of all target animals estimated to be present, which rarely occurs under actual field conditions. The following parameters and assumptions were used to estimate WS take with diphacinone baits for each target rodent species:

- **Northern and Botta's Pocket Gophers**: These pocket gopher species are very territorial and live solitary lives except during breeding and when mothers are with young. They generally have about 4 young per litter and 1 litter per year in the north and 2 litters in the south part of their range, where they may not hibernate in the winter. WS take occurred in New Mexico. Young are weaned in 3–4 weeks and quickly leave the burrow afterward. Average weights equal 3.8 oz. (108 g) for northern pocket gophers and 5.5 oz (156 g) for Botta's pocket gophers. Pocket gophers can consume 50% or more of their body weight per day. If they consume 20% of a bait block over two days, five northern pocket gophers and four Botta's pocket gophers would be taken per one 4-ounce bait block. Both species occur at densities up to 8 per acre in high-density situations, but this risk assessment assumed that 4 pocket gophers were taken per acre treated on average.

- **Old World Invasive Rodents:** Old World rodents, invasive throughout the United States, are common, especially in urban areas and ports as well as islands. Old World rodents include the house mouse and brown, black, and Pacific rats. These species are highly commensal and live in loose to dense colonies in burrows, structures, and tree canopies (especially black and Pacific rats). Rats eat more grams of food per day, but for estimating take, it was assumed that rats consume 6% and house mice 12% of their body weight per day from treated baits. Body weights on average are 340 g, 153 g, 71 g, and 23 g for brown, black, and Pacific rats and house mice, respectively. Therefore, this risk assessment assumed that 20 brown rats, 45 black rats, 96 Pacific rats, and 148 house mice were taken per pound of bait applied (including 10% wastage, i.e., bait destroyed or not consumed).

[4] MIS - Computer-based Management Information System used for nationally tracking WS wildlife damage management methods used and activities. Methods and activities are tracked such as diphacinone projects but take is not always estimated.

- **Small Indian Mongoose:** This small invasive mongoose species is native to Iraq and northern South Asia but has been introduced to many regions of the world, including the Caribbean and Pacific Islands. They were introduced to Caribbean Islands to kill rats in sugar cane and later to Hawaii. Without predators, they colonized islands in high densities and caused problems for native wildlife. Mongooses are weasel-like in body shape, agile, adept at climbing, and hunt any time, day or night. They can weigh up to 900 g (2 lbs). Gestation is 49 days, with litter sizes ranging from 1–4 pups. They have two to three litters per year. Young mongooses are weaned and begin hunting at six weeks. The pups stay with their mother until they are sexually mature at 4 to 6 months. Life expectancy is 4–5 years. It should be noted that baits designed for rodents are generally not preferred by mongooses and result in lower mortality rates (Sugihara et al. 2018). Thus, we estimated comparatively low bait consumption rates (10% of bait applied) by mongooses during rodent and mongoose baiting projects that used Ramik® Mini Bars All-Weather Bait. Given their sensitivity to diphacinone (acute oral LD_{50} [5] = 0.18 milligrams diphacinone per kilogram bodyweight (mg/kg-bw) (Keith et al. 1989), this risk assessment assumed that one mongoose was taken for every two 1-oz. baits that were consumed by mongooses (rather than rodents). In the future, take may be higher with a new soft diphacinone meat bait that WS is developing with higher palatability for mongooses (Antaky et al. 2023).

Overall, WS applied four different diphacinone baits (under five labels) in four states and one U.S. territory (Table 2a) from FY11 to FY20 to control Old World rodents and mongooses to protect sensitive species and pocket gophers to protect turf, alfalfa, and rangeland. An additional bait label was used by WS in FY21, but minimally. In addition to the species taken, other species that can be controlled with diphacinone are listed in Table 1.

Estimates of the annual average number of target rodents and mongooses killed with diphacinone baits by WS in wildlife damage management activities and the annual average amounts of diphacinone baits used from FY11 to FY15 and from FY16 to FY20 throughout the United States are provided in Table 2a. Between FY11 and FY15, WS used an annual average of 177 pounds of diphacinone baits to take an annual estimated average of 72 pocket gophers, 10,990 invasive Old World rodents, and 120 invasive mongooses. Between FY16 and FY20, WS used an annual average of 18 pounds of diphacinone baits, a much-reduced usage, to take an annual estimated average of 23 pocket gophers and 378 brown rats. All applications for gophers were belowground directly into the burrow, for Old World rodents in terrestrial bait stations. These products were applied in four states and one U.S. territory, including Hawaii, Massachusetts, New Mexico, Virginia, and the U.S. Virgin Islands (Table 2a). While it is likely that some nontarget animals were taken, no known nontarget animals were found. For WS projects, the most likely nontarget animals that would be taken would be nontarget rodents in gopher burrows following their removal, as gophers are very aggressive towards other animals in their burrows while they are active. The other projects were on islands where nontarget mammals would not be present, and baits were not broadcast, minimizing potential bird take.

WS also sold Eaton's Gopher Blocks to certified pesticide applicators, but minimally at 4.5 lbs. per year from FY11–FY20 (Table 2a,b). This diphacinone product was only sold in New Mexico.

[5] LD_{50}, or lethal dose, is the amount of a chemical given all at once that kills 50% of a population of test animals

Table 2a. Annual Average Target Animal Take and Diphacinone Products Applied by WS from FY11 to FY15 and FY16 to FY20.

Species	FY11-15 Take[A]	FY11-15 Lbs Used	FY16-20 Take[A]	FY 16-20 Lbs Used	USEPA Registration Number	State
Northern Pocket Gopher	-	-	5	0.2	56-57	NM
Botta's Pocket Gopher	72	4.5	18	1.3	56-57	NM
House Mouse*	4,440	30.0	-	-	HI-980005	HI
Black Rat*	76	2.4	-	-	56228-35	VI
Black Rat*	4,056	90.5	-	-	HI-980005	HI
Brown Rat*	98	4.9	258	12.9	56228-35	MA
Brown Rat*	150	7.5	-	-	HI-980005	HI
Brown Rat*	-	-	120	3.8	56-42	VA
Pacific Rat*	2,160	22.5	-	-	HI-980005	HI
Small Indian Mongoose*	120	15.0	-	-	HI-980005	HI
Total (8 spp.)	**11,172**	**177.3**	**401**	**18.2**		**4 States, 1 Terr.**

[A]Target animals estimated to have been taken. If the WS applicator did not estimate take, the number of target animals taken was estimated. Only black rats and Botta's pocket gophers had a few projects with take estimated by WS specialists for FY11-FY15, but most take was for brown rats and Botta's pocket gophers for FY16-FY20.
*Introduced species SLN = Special Local Need registration

Table 2b. Annual Diphacinone Products Sold by WS from FY11 to FY15 and FY16 to FY20.

Product Name	FY11-15 Lbs Sold	FY 16-20 Lbs Sold	USEPA Registration Number	State
Eaton's Gopher Bait Block	6	2.9	56-57	NM

2 PROBLEM FORMULATION

The following sections discuss physical and chemical properties, environmental fate, and hazard identification for diphacinone.

2.1 Physical and Chemical Properties

Diphacinone (synonym: 2-(diphenylacetyl)indan-1,3-dione; chemical formula: $C_{23}H_{16}O_3$; CAS No.: 82-66-6) is an organic compound with a molecular weight of 340.48 and a molecular structure shown in Figure 1 (USEPA 2015e, NIH 2023).

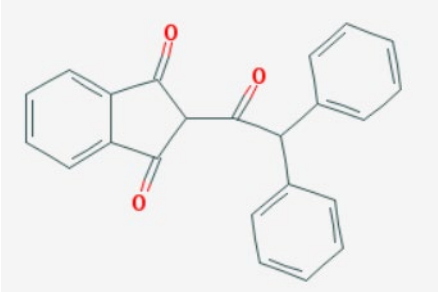

Figure 1. Chemical structure for diphacinone

Diphacinone is a yellowish-to-whiteish powder at room temperature (NIH 2023). It has a low vapor pressure of 1.03×10^{-10} mm Hg at 25°C and Henry's Law constant of 2×10^{-10} atm-m^3/mol (USEPA 2015e, NIH 2023). It has a low water solubility of 0.3 milligrams (mg)/Liter (L) (USEPA 2015e). Diphacinone has an octanol-water partition coefficient (K_{ow}) of 20,000 and an estimated adsorption coefficient (K_{oc}) between 1,700 milliliters (ml)/gram (g) and 2,100 ml/g (USEPA 2015e).

2.2 Environmental Fate

Environmental fate describes the processes by which chemicals move and degrade in the environment. The environmental fate processes include 1) persistence, degradation, and mobility in soil; 2) movement to air; 3) migration potential to groundwater and surface water; 4) degradation in water; and 5) plant uptake.

Due to the label-mandated use, where applied, diphacinone will be present in environmental media. Due to low vapor pressure, diphacinone released in the air will remain in the particulate phase at 25°C and be removed from the atmosphere by wet and dry deposition (NIH 2023). Diphacinone has a low water solubility with a low potential for migration to groundwater via leaching or to surface water via runoff (USEPA 2015e). The K_{ow} (20,000) indicates that there is a potential for bioconcentration in aquatic biota (USEPA 2015e). Diphacinone was stable against hydrolysis at pH 7 and 9 but underwent hydrolysis at pH 5 with an extrapolated half-life of 44 days (USEPA 2015e). Although diphacinone is stable between pH 7 and 9 and will adsorb to suspended solids and sediment in the water, it has been observed in water to undergo degradative photolysis.

Diphacinone is only slightly mobile in soil based on its K_{oc} (USEPA 2015e). Calculated K_{oc} values range from 1,700 to 2,100 (Log K_{oc} from 3.24 to 3.32). Diphacinone is immobile in tightly packed soil in laboratory settings and will reside in the top 3 inches. Diphacinone is unlikely to volatilize from moist soils or water due to a Henry's Law constant of 2×10^{-10} atm-m^3/mol (USEPA 2015e). The biodegradation half-life for diphacinone was 28.3 to 31.7 days in sandy loam soil.

2.3 Hazard Identification

The following section summarizes available acute and chronic toxicity and the experimental exposure data used to evaluate the hazards of diphacinone to human health and nontarget mammals. On an acute basis, technical diphacinone is a hazard to human health and can be lethal due to acute oral, inhalation, and dermal toxicity. The end-use bait formulations containing 0.005% w/w diphacinone have low acute oral and dermal toxicity. USEPA waived the data requirement for acute inhalation toxicity for the waxy pelleted and block registered products due to the exposure route not being relevant (Bell Laboratories 2020). However, a recent EPA review concluded that users of "loose" pellet products containing diphacinone may have inhalation exposures (USEPA 2020a).

2.3.1 Mechanism of Action and Metabolism

Diphacinone is a first-generation anticoagulant rodenticide (USEPA 2015a;2020b). Diphacinone toxicity is manifested as an increase in prothrombin and partial thromboplastin time (USEPA 2015c). Signs of toxicity include dyspnea, lethargy, hemorrhage from the nose, and uterine bleeding (USEPA 2015c). Death follows excessive external or internal bleeding (USEPA 2015c).

Diphacinone absorption peaked in rats at 4 hours after administration. Distribution data following ingestion shows that the liver (14–25% of the dose), muscle (0.18–4.4% of the dose), fat (0.55–1.16% of the dose), and lung (0.04–0.5% of the dose) were the tissues with the most significant retention of diphacinone. Elimination of diphacinone was primarily in feces (47–77% of the dose in rats and 69–73% of the dose in mice) (USEPA 1998).

2.3.2 Human Incidents

USEPA (2008) published the Risk Mitigation Decision (RMD) for ten rodenticides to minimize exposure to children and ecological systems, including wildlife. To minimize children's exposure to rodenticide products used in homes, USEPA (2008) required that all rodenticide bait products marketed to general and residential consumers (packages containing ≤1 lb. of bait) be sold only with bait stations, with loose bait (e.g., pellets and meal) prohibited as a bait form. All "professional" products containing FGARs, which includes all products containing the active ingredients warfarin, chlorophacinone, and diphacinone, had to be sold in containers of ≥4 pounds net contents to discourage non-professional (general residential consumer) use.

In 2015, USEPA (2015c) reviewed the Incident Data System (IDS), American Association of Poison Control Centers (AAPCC), and the Annual Report for rodenticide incident trends. From 2011, the first year of mandatory compliance with USEPA (2008) RMD, to 2014, there was a 17% reduction in overall rodenticide exposure to children under six years old. The USEPA Health Effects Division (HED) completed an updated analysis of exposure incidents for eleven anticoagulant and non-anticoagulant rodenticides (USEPA 2020a). As per the USEPA HED (USEPA 2020a), IDS and AAPCC data suggest that the overall frequency of rodenticide incidents appears to be decreasing over time, and the total number of reported child exposures was reduced by 46% between 2011 and 2017 (USEPA 2020a). While the IDS reported a 60% increase in FGAR incidents between 2009 and 2018, the total number of incidents was low (10 FGAR incidents in 2009 and 52 in 2018) (USEPA 2020a;c). Also, the number of AAPCC-reported FGAR incidents declined in the same period. USEPA (2020a) concluded that the IDS and AAPCC data suggest that overall FGAR exposure trended downward after the publication of the 2008 RMD. In addition, data from the IDS suggests that reported diphacinone incidents to humans, domestic animals and wildlife have decreased since 2014 (USEPA 2023c)[6].

A retrospective occupational exposure analysis of the National Institute for Occupational Safety and Health Sentinel Event Notification System for Occupational Risk (SENSOR)-pesticides (2011-2015), the California Pesticide Illness Surveillance Program (PISP) (2012-2016), and IDS (2015-2019), conducted by the HED reported 21 occupational exposure incidents to rodenticides (USEPA 2020a). Further review indicated that most exposures reported to SENSOR and PISP databases involved the manual application of zinc phosphide-containing products and were low in severity. However, one severe occupational exposure case involved diphacinone and a pregnant employee driving a company vehicle with diphacinone bait glued to the vehicle's interior, which was an illegal product use. The woman experienced headaches and nausea, and the neonate was born with hemolytic anemia.

[6] USEPA does not guarantee the completeness or adequacy of the contents of the IDS USEPA. 2023c. Pesticide Registration Incident Data System US Environmental Protection Agency https://ordspub.epa.gov/ords/pesticides/f?p=359:5:::::: Accessed 08/07/2023..

2.3.3 Toxicity

Technical-grade diphacinone has high acute toxicity via oral, dermal, and inhalation routes of exposure (Toxicity Category I). Technical diphacinone is a moderate irritant to the eye (Category III) but is not irritating to the skin (Category IV) and is not a dermal sensitizer (USEPA 1998;2015c) (Table 3a).

Table 3a. Acute technical grade diphacinone toxicity data for mammals.

Test/Species	Toxicity Value	Toxicity Category	Reference
Oral LD_{50}[1] (rat)	2.5 mg/kg-bw (male)	I	(USEPA 2015c)
Oral LD_{50} (rat)	2.1 mg/kg-bw (female)	I	(USEPA 2015c)
Oral LD_{50} (rat)	2.3 mg/kg-bw (combined)	I	(USEPA 2015c)
Dermal LD_{50} (rabbit)	3.6 mg/kg-bw (male)	I	(USEPA 2015c)
Inhalation LC_{50}[1] (rat)	<6 µg/L (male/female)	I	(USEPA 2015c)
Eye irritation (rabbit)	Moderate irritation clearing by day 4	III	(USEPA 2015c)
Dermal irritation (rabbit)	Slight erythema clearing within 48 hours, but 4/6 rabbits died between 8 and 10 days	IV	(USEPA 2015c)
Dermal sensitization (guinea pig, *Cavia porcellus*)	Neither a dermal irritant nor a sensitizer at a non-lethal dose level (2.5 mg/day)	na	(USEPA 2015c)

[1]LD_{50} (median lethal dose) and LC50 (median lethal concentration) is a toxicity measurement for the dose or concentration (airborne) of a substance necessary to kill 50% of a population

Table 3b. Acute toxicity data for end-use bait product containing 0.005% w/w (50 ppm) diphacinone in mammals.

Test/Species	Toxicity Value	Toxicity Category	Reference
Oral LD_{50} (rat)	>5,000 mg/kg-bw	IV	(Bell Laboratories 2020)
Dermal LD_{50}	>5,000 mg/kg-bw	IV	(Bell Laboratories 2020)
Inhalation LC_{50}	Waived	Na	(Bell Laboratories 2020)
Eye irritation	Not an irritant	IV	(Bell Laboratories 2020)
Dermal irritation	Not an irritant	IV	(Bell Laboratories 2020)

2.3.4 Acute Toxicity

Oral Toxicity

An acute toxicity study (5/sex/dose level) to determine the lethal dose for 50% (LD_{50}) of diphacinone to Spartan rats was conducted at doses 0, 0.79, 1.25, 1.98, 3.15, 5.00, 7.94, 12.60, 20.01, 31.76, 50.40, or 201.7 mg/kg-bw/day (USEPA 1998). Symptoms of exposure were clear or colored nasal discharge, soft stool, diarrhea, decreased motor activity, occasional dry cornea at lower doses, lacrimation (excessive tearing), ataxia (impaired muscle movement function), cyanosis (bluing of the skin), and orifice/body cavity hemorrhage at higher doses. Mortalities occurred at 1.25 mg/kg-bw/day (1/5 female), and 100% mortality for both sexes occurred at 5 mg/kg-bw/day. The LD_{50} for male rats was 2.50 mg/kg-bw/day, and for female rats, the LD_{50} of 2.31 mg/kg-bw/day was established for this study. In conjunction with others, these representative exposure studies place diphacinone into oral Toxicity Category I (USEPA 1998;2015c). An additional study exposed male and female Sprague-Dawley rats 5/sex/dose level) to technical grade diphacinone (99.0%) at 0.13, 0.20, 1.0, or 2.5 mg/kg-bw (USEPA

1998). The lowest observed adverse effect level (LOAEL) from the single-dose administration was 0.20 mg/kg-bw based on increased activated partial thromboplastin time in female rats. The no observed adverse effect level (NOAEL) was 0.13 mg/kg-bw.

Dermal Toxicity

New Zealand white rabbits were dermally exposed to 0, 1, 5, 10, 25, or 50 mg/kg-bw for 24 hours. Male rabbits (n=1) at all doses died (USEPA 1998). Female rabbits at the three highest doses died. Mortalities occurred days 7-13 post-exposure, and symptoms of exposure were hemorrhage, discoloration of organs, somnolence (sleepiness), loss of fluids, absence of feces, and vasoconstriction. The estimated LD_{50} for dermal exposure to diphacinone is 3.6 mg/kg-bw. These data place dermal exposure to diphacinone into the Toxicity Category I (USEPA 2015c).

Inhalation

In a range-finding inhalation study (1/sex/dose level), male Sprague-Dawley rats exposed for 1 hour to 0, 0.01, 0.11, or 1.1 mg per liter (mg/L) of diphacinone died (USEPA 1998). Two female rats survived to the end of the 7-day observation period. Symptoms observed were hemorrhage in the thoracic cavity, cranial cavity, and various organs. To determine the lethal concentration of airborne diphacinone, a follow-up study was conducted in Sprague-Dawley rats (5/sex/dose level) exposed to 4 hours of a time-weighted average aerosol of 6 micrograms per liter (µg/L) and observed for 14 days (USEPA 1998). Mortality occurred on day 4–8 in 5/5 males and 4/5 female rats. Based on the observed LC_{50} of <6 µg/L in rats, inhalation exposure to diphacinone is in a Toxicity Category of I (USEPA 2015c).

Eye and Dermal Irritation and Sensitization

New Zealand white rabbits (n=3) were exposed to 0.1 g diphacinone (98%) via eye irrigation for 20–30 seconds (USEPA 1998;2015c). No corneal opacity was observed. Technical grade diphacinone is in the Toxicity Category III for eye irritation. The acute systemic toxicity of diphacinone complicated dermal irritation and sensitivity studies. However, it was determined that non-lethal concentrations of diphacinone are neither a dermal sensitizer nor an irritant (USEPA 1998;2015c).

2.3.5 Sub-chronic/Chronic Toxicity

Oral: In a 14-day oral toxicity study, Sprague-Dawley rats (5/sex/dose level) were orally gavaged technical grade diphacinone (99.0%) at 0, 0.025, 0.040, 0.085, or 0.175 mg/kg-bw/day (USEPA 1998;2015c). No clinical signs of toxicity were observed at doses of 0.025, 0.040, or 0.085 mg/kg-bw/day. At 0.175 mg/kg-bw/day, dyspnea, lethargy, hemorrhage from the nose, and ptyalism (overproduction of saliva) increased. At the 0.175 mg/kg-bw/day exposure, all female (5/5) and 3 of the 5 male rats died by treatment day 11. The LOAEL and NOAEL for the 14-day study were 0.085 and 0.040 mg/kg/day, respectively.

Dermal Toxicity

In a 21-day dermal toxicity study, rabbits were dermally exposed to diphacinone at dosage levels of 0, 0.1, 1.0, or 10.0 mg/kg-bw/day (USEPA 1998). Two males and females in each group had doses applied to abraded skin. The subchronic NOAEL was 0.1 mg/kg-bw/day, and the LOAEL was 1.0 mg/kg-bw/day, based on mortality accompanied by clinical signs of anticoagulant activity

(USEPA 1998). No chronic studies testing have been conducted due to the non-food uses for diphacinone.

2.3.6 Developmental and Reproductive Effects

Pregnant Sprague-Dawley rats (25/group) were exposed to 0, 0.010, 0.025, or 0.075 mg/kg-bw/day for 9 days (gestational days 6–15) (USEPA 2015c). Adverse effects were observed in all treatment groups. Based on clinical signs consistent with anticoagulant activity (reddish urogenital staining and reddish fluid in cage), a maternal NOAEL could not be established (NOAEL <0.010 mg/kg-bw/day). No effects were reported on body weight, food consumption, or histopathology. Although compound-related altered growth and/or developmental abnormalities were not observed, there were increased early fetal resorptions and increased fetal resorptions per animal at 0.075 mg/kg-bw/day. The developmental LOAEL was determined to be 0.075 mg/kg-bw/day, and the developmental NOAEL was 0.025 mg/kg-bw/day. The prenatal developmental toxicity study in rabbits and a reproduction and fertility toxicity study in rats was not required by the Hazard and Science Policy Council (USEPA 2015d).

2.3.7 Neurotoxicity and Immunotoxicity Effects

Although diphacinone has been observed to exert overt acute anticoagulant activity, potentially leading to severe injury or death, neurotoxicology and immunology sensitivity studies do not indicate these are a concern (USEPA 1998;2020a). As a result, the Hazard and Science Policy Council has not required neurotoxicity studies and waived immunotoxicity studies for diphacinone registration, and determined that available studies adequately characterize toxicity for any quantitative risk assessments (USEPA 2015c). A literature review resulted in similar exposure sensitivity conclusions to other FGARs with similar modes of action (Kataranovski et al. 2003).

2.3.8 Carcinogenicity and Mutagenicity

Diphacinone is not considered to be a carcinogen, although USEPA did not require carcinogenicity studies for diphacinone due to its non-food uses (USEPA 2020a). Available studies do not indicate mutagenicity for diphacinone (USEPA 2015c;2020a).

2.3.9 Endocrine System Effects

An Endocrine Disruptor Screening Program (EDSP) was developed to characterize endocrine activity in commercial products, pesticides, and environmental contaminants (USEPA 2023a). EDSP uses a two-tier risk characterization approach consisting of screening candidate compounds for estrogen, androgenic, and thyroid receptor activity and quantifying their impact on environmental and human health (USEPA 2023a). Before 2012, Tier 1 screening involved five *in vitro* and six *in vivo* assays (Browne et al. 2015). To address the growing need for a more rapid but equally comprehensive review of thousands of candidate compounds, the EDSP revised Tier 1 screening to include computational endocrine activity models and high-throughput assays. Tier 2 testing data characterizes the endocrine-related health effects, dose response, and health risks of candidate compounds and substances. Although Diphacinone is listed in the EDSP Universe of Chemicals (USEPA 2012;2023a). Diphacinone was not screened for estrogen receptor bioactivity.

2.3.10 Toxicity of Other Ingredients

The other 99.995 percent of all diphacinone product formulations are other ingredients (e.g., (USDA APHIS 2009, HACCO 2021). The identity and safety profile of non-active ingredient contents of diphacinone formulations is not presented on labels or safety data sheets (HACCO 2021). The other or “inert” ingredients listed on pesticide labels are considered confidential business information and not generally available to the public. However, they still must be approved inert ingredients for non-food use pesticides by USEPA (2023b).

3 DOSE-RESPONSE ASSESSMENT

3.1 Human Health Dose-Response Assessment

A dose-response assessment evaluates the dose levels (toxicity criteria) for potential human health effects. A 14-day diphacinone oral exposure study in rats reported a NOAEL of 0.085 mg/kg-bw/day and LOAEL of 0.175 mg/kg-bw/day based on a critical effect of increased prothrombin time and activated partial thromboplastin time for repeated doses (USEPA 2015c). A 21-day oral study in mice reported a NOAEL of 0.5 mg/kg-bw/day and a LOAEL of 1 mg/kg-bw/day based on hemorrhages and external bleeding (USEPA 2015c). A 21/28-day dermal study in rabbits using diphacinone technical grade reported a NOAEL of 0.1 mg/kg-bw/day and LOAEL of 1 mg/kg/day based on hemorrhagic lesions in tissues/organs (USEPA 2015c).

Maternal diphacinone toxicity studies in pregnant Sprague-Dawley rats were inconclusive. A NOAEL for maternal effects could not be established due to adverse effects at every exposure level (NOAEL <0.010 mg/kg-bw/day) (USEPA 1998). Based on the increased incidence of fetal resorptions, the developmental NOAEL and LOAEL were observed to be 0.025 and 0.075 mg/kg-bw/day, respectively (USEPA 1998).

USEPA did not include toxicology studies with mice as an experimental model in determining the point of departure in their risk assessment for diphacinone (USEPA 2015c). The USEPA reported that "the mouse is considerably less susceptible to the toxic effects of diphacinone than other mammalian species” (USEPA 1998). Studies involving rabbit models have been evaluated but were not selected in determining the point of departure due to sample size limitations and increased tolerance to diphacinone (Correll et al. 1952). Therefore, for this risk assessment and to protect the most vulnerable of the population that is at the highest risk of injury from exposure, the prenatal rat developmental evaluation (USEPA 1998) was chosen as the critical study with the critical effects of anticoagulant activity and increased fetal resorptions determining the point of departure (NOAEL of 0.025 mg/kg-bw/day).

An uncertainty factor is a human health risk assessment conceptual tool predicated on the assumption that a biological response increases or decreases in relation to increasing or decreasing exposure to a biologically active compound. If a sufficient reduction in exposure from the first known boundary of deleterious effects can be accomplished, then a level of exposure protective of populations, including the vulnerable, can be determined (Dankovic et al. 2015). Uncertainty factors are used to address the differences between experimental data and actual human health effects. These include uncertainty factors for interspecies and intraspecies differences, differences in exposure duration, and data quality issues. For this risk assessment, uncertainty factors of 10 and $\sqrt{10}$ were applied to the NOAEL to account for genetic variability between humans and the variability between animals and humans. To accommodate for the absence of male rodents in the reference study, an additional factor of $\sqrt{10}$ was applied to the

uncertainty extrapolation for a total uncertainty factor of 100. The application of uncertainty factors to the point of departure results in a reference dose of 0.25 µg/kg-bw/day.

Diphacinone has only non-food uses. Thus, USEPA (2015c) did not establish food tolerances or conduct a dietary exposure assessment.

3.2 Ecological Effects Analysis

This section summarizes available diphacinone toxicity data for aquatic and terrestrial species.

3.2.1 Aquatic Effects Analysis

Diphacinone has moderate acute oral toxicity to freshwater fish and invertebrates (USEPA 2015e) (Table 4). Toxicity data is lacking for estuarine and marine fish, invertebrates, and aquatic plants (USEPA 2015e).

Table 4. Diphacinone toxicity in aquatic species.

Test Species	Scientific Name	LC_{50}/EC_{50} (mg a.i./L)	Reference
Rainbow Trout	*Oncorhynchus mykiss*	96-hr LC_{50} = 2.6 (moderately toxic)	(USEPA 2015e;2020b)
Bluegill	*Lepomis macrochirus*	96-hr LC_{50} = 7.5 (moderately toxic)	(USEPA 2015e)
Water Flea	*Daphnia magna*	48-hr EC_{50} = 1.8 (moderately toxic)	(USEPA 2015e;2020b)

3.2.2 Terrestrial Effects Analysis

3.2.2.1 Mammals

Technical diphacinone has very high toxicity to mammals on an acute oral and dietary basis (Table 5) (USEPA 2015e;2020b). In laboratory brown rats, the acute oral LD_{50} was 1.9 mg/kg-bw, and the acute dietary LC_{50} was 2.08 mg/kg-diet. Small Indian mongoose are highly susceptible to diphacinone at 0.18 mg/kg-bw (Keith et al. 1989). Marsh (1985) (cited in (USEPA 2004)) calculated an acute oral LD_{50} of 85 g of diphacinone bait for a 10-lb dog. No chronic toxicity studies are available.

Table 5. Diphacinone toxicity in mammals.

Test species	Scientific Name	Test	Reference
Laboratory (Brown) Rat	*Rattus norvegicus*	Acute oral LD_{50} = 1.9 mg/kg-bw (very highly toxic)	(USEPA 2015e;2020b)
Laboratory (Brown) Rat	*Rattus norvegicus*	Acute dietary LC_{50} = 2.08 mg/kg-diet, 5-day exposure (very highly toxic)	(USEPA 2015e)
Domestic Cat	*Felis catus*	LD_{50} = 15 mg/kg-bw	(Eisemann and Swift 2006)
Domestic Dog	*Canis domesticus*	LD_{50} = 2 to 3 mg/kg-bw	(Eisemann and Swift 2006)
Woodland [Pine] Vole	*Microtus pinetorum*	LD_{50} = 67.7 mg/kg-bw	(Byers 1978)
Meadow Vole	*Microtus pennsylvanicus*	LD_{50} = 11.7 mg/kg-bw	(Byers 1978)
Common Vampire Bat	*Desmodus rotundus*	LD_{50} <0.91 mg/kg-bw	(Eisemann and Swift 2006)
Small Indian Mongoose	*Herpestes javanicus* [Synonym: *Urva auropunctata*]	LD_{50} = 0.18 mg/kg-bw	(Keith et al. 1989)

Target and nontarget mammals that ingest sufficient amounts of diphacinone-treated bait to be fatal may take several days to die from toxicity. Due to this delay in symptoms, animals may continue to eat available bait, ingesting more than a sufficient dose to cause toxicity. The elimination half-life for diphacinone in mammals, based on elimination rates from the liver, is 12.4 days (USEPA 2020b).

3.2.2.2 Birds

Birds are more tolerant of diphacinone than mammals. In birds, diphacinone is moderately toxic to practically nontoxic on an acute oral basis (Table 6) (USEPA 2020b). Diphacinone is moderately toxic (LD_{50} of 96.8 mg/kg-bw) to the American kestrel, which is more sensitive than the standard test species of northern bobwhite and mallard (Rattner et al. 2011). USEPA used the acute oral data for northern bobwhite as an endpoint in their risk assessment (referenced below under the exposure assessment and risk characterization) (USEPA 2020b).

On an acute dietary basis, diphacinone is moderately toxic with an LC_{50} of 906 mg/kg-diet in mallards and slightly toxic in the northern bobwhite with an LC_{50} of 5,000 mg/kg-diet (USEPA 2020b). No chronic avian studies are available for diphacinone; however, to be conservative, USEPA used a chronic toxicity study for chlorophacinone to represent chronic toxicity for diphacinone, since chlorophacinone is more toxic to birds than diphacinone (USEPA 2020b). Chlorophacinone reduced the mean 14-day survivor weights in mallards, with a NOAEC of 0.046 mg/kg-diet and LOAEC of 0.096 mg/kg-diet (USEPA 2020b).

Table 6. Diphacinone toxicity in birds.

Test species	Scientific Name	Test	Reference
Northern Bobwhite	*Colinus virginianus*	Acute oral LD_{50} = 1,630 mg/kg-bw (slightly toxic) Acute dietary LC_{50} = 5,000 mg/kg-diet (slightly toxic)	(Eisemann and Swift 2006, USEPA 2015e;2020b)
American Kestrel	*Falco sparverius*	Acute oral LD_{50} = 96.8 mg/kg-bw, 7-day test (moderately toxic)	(USEPA 2015e)
Mallard	*Anas platyrhynchos*	Acute oral LD_{50} = 3,160 mg/kg-bw (practically nontoxic) Acute dietary LC_{50} = 906 mg/kg-diet, 25-day test (moderately toxic)	(USEPA 2004;2015e)

3.2.2.3 Reptiles and Amphibians (terrestrial phase)

In the brown tree snake, diphacinone was highly toxic with an acute oral (single dose) LD_{50} of 20.75 mg/kg-bw (USEPA 2015e).

3.2.2.4 Terrestrial Invertebrates and Microorganisms

Limited information exists on the toxicity of diphacinone to invertebrates. In one study, diphacinone residues were quantified in snails and slugs fed diphacinone baits for 7 days in the laboratory or collected on or near diphacinone baits in the field (Johnston et al. 2005). Although diphacinone residues were detected in laboratory and field-collected glass snails (*Oxychilus* spp.), great gray slugs (*Limax maximus*), and marsh slugs (*Deroceras laeve*), no mortality occurred, indicating acute toxicity of diphacinone to snails and slugs is likely minimal.

3.2.2.5 Terrestrial Plants

Toxicity data is unavailable for terrestrial plants (USEPA 2015e).

4 EXPOSURE ASSESSMENT

4.1 Human Health Exposure Assessment

Diphacinone-50 Conservation (a pellet product) is a restricted use pesticide, and only certified applicators or persons under their direct supervision who are employees or under the direct supervision of federal agencies responsible for wildlife management may use the product (USEPA 2019a). The other four diphacinone block products used by WS are general use pesticides that the public and WS can use without a certified pesticide applicator's license.

Exposure assessments estimate the potential exposure of humans to diphacinone. An identified exposure pathway for diphacinone includes (1) a release from a source, (2) an exposure point where contact can occur, and (3) an exposure route such as ingestion, inhalation, or dermal contact by which contact can occur (USEPA 1989). Exposures for the identified human populations are qualitatively evaluated for each identified exposure pathway.

4.1.1 Identification of Potentially Exposed Human Populations and Complete Exposure Pathways

Based on the expected WS use pattern and label restrictions for diphacinone applications, workers applying the rodenticide baits in the field are the most likely subgroup of the human population to be exposed to diphacinone. Exposure during transportation is not anticipated because the material is sealed. The baits are ready to use with no mixing required. Diphacinone-50 Conservation is a pelleted bait; the other four are bait blocks. The label application methods include bait stations, burrow baiting, aerial and ground broadcast baiting, and canopy baiting, with broadcast baiting having the greatest potential for exposure. Broadcast aerial and ground baiting of Diphacinone-50 Conservation pellets are usually permitted only on islands uninhabited by humans. Although the potential for exposure from the proposed WS use pattern is low, accidental exposure may occur during application. The risk of exposure via ingestion or inhalation is deemed to be minimal due to outdoor use and label-mandated application restrictions and personal protective equipment (PPE). Block products cannot be applied using broadcast application methods.

Applicators and other handlers must wear long pants, shoes plus socks, and barrier laminate gloves. Any person who retrieves carcasses or unused bait following the application of diphacinone must wear barrier laminate gloves. Following label directions, including the use of proper PPE, will minimize worker exposure to diphacinone via inhalation and dermal contact routes.

A significant direct exposure pathway to Diphacinone-50 Conservation bait is not identified for the public because this product is a restricted use pesticide and can only be sold to certified applicators that are employees of federal agencies responsible for wildlife management for conservation uses on islands. Diphacinone-50 Conservation can only be used by a certified applicator or persons under their direct supervision. Belowground burrow baiting is only permitted in uninhabited non-crop areas, and bait bolas or sachets must only be applied to tree canopies or uninhabited grounded vessels or vessels in jeopardy of being grounded. Manual or aerial

broadcast applications of Diphacinone-50 Conservation are prohibited by the label in areas of human habitation unless allowed by USEPA for specific island projects under supplemental labels.

General use block formulations of diphacinone and Diphacinone-50 Conservation pellets used aboveground in areas that are accessible to children and pets must be applied in tamper-resistant bait stations. Tamper-resistant bait stations also minimize post-application exposure in adults. As a result of these use restrictions for diphacinone products used by WS, the public is not considered a vulnerable population for direct exposure to diphacinone used by WS (see Section 2.1).

Although oral exposure to all formulations of diphacinone is acutely hazardous, label restrictions render accidental dietary exposure an incomplete exposure pathway. Diphacinone has a low potential for volatilization due to its low vapor pressure. It will adsorb readily to organic material in the soil, minimizing inhalation risks and mobility into nearby water bodies or aquifers. In addition, label restrictions state that no applications are allowed directly to water, areas where surface water is present, or intertidal areas below the mean high-water mark (USEPA 2019a). As a result, surface and groundwater exposure pathways are also incomplete.

4.1.2 Occupational Exposure Evaluation

This section quantitatively evaluates worker exposures from an incidental direct contact pathway while applying baits. Under the accidental exposure scenario, it assumes that chemical-resistant gloves are broken.

The estimates of exposure to certified applicators using diphacinone are based on surrogate study data available in the Pesticide Handlers Exposure Database (PHED) (USEPA 2021a). The "trap/bait station refillable" or "applicator granules by hand" exposure scenario in the PHED table is the closest scenario representing potential exposure for the application method on the label for pellets and sachets containing diphacinone at 0.005%. Under this exposure scenario, the exposure unit for dermal contact under the single layer (long-sleeve shirt, long pants, shoes plus socks) and single and double pair of gloves is 71 and 40.3 mg/lb ai, respectively. The exposure unit for a scenario where a WS applicator lacks or dons inadequate hand protection, such as broken gloves, is 104 mg/lb ai. Lower and upper application use rates were estimated based on 4 to 16 ounces (113 to 454 g) of bait per treatment location with placements at an interval of 16 to 82 feet (5 to 25 meters) per label directions to bait rats (USEPA 2016).

Other conservative assumptions used for this exposure evaluation include an accidental dermal contact of 1% pelleted baits when PPE integrity is compromised without replacement for 8 hours. A worker spends a half-hour at each placement. The following equations are used to estimate the exposure dose of direct contact for workers:

Exposure Dose = Daily Dose Rate/Body Weight

Daily Dose Rate = Unit Exposure (mg/lb ai) x Application Rate (lb ai/acre) x Area Treated (acre/day) x Dermal Absorption Rate (%)

Exposure doses were estimated for the application rates specified on the labels and summarized in Appendix A.

4.2 Ecological Exposure Assessment

WS has specific use patterns for diphacinone. WS uses diphacinone to eradicate or manage invasive rodents on islands for conservation purposes; house mice, Pacific rats, and black rats in Hawaii; Norway rats on islands in Massachusetts; and pocket gophers in New Mexico to protect lawns, landscaping, and pecan trees.

The application method will affect the primary and secondary exposure potential of nontarget species. Depending on the formulation used for treatment, the label may allow for applications belowground by hand, bait bolas, in tamper-resistant bait stations, or through aerial and ground broadcast applications. WS applied baits in bait stations or underground in gopher burrows from FY11–FY20.

The USEPA's ecological risk assessments (USEPA 2004;2020b) calculate the risk quotient (RQ) for different species groups and compares this to the regulatory levels of concern (LOC) to understand the potential exposure risk associated with the use of a pesticide. The RQ is calculated by dividing an estimated environmental concentration by a toxicity endpoint and determines if the estimated environmental concentration is above or below the concentration with an effects endpoint. The vertebrate LOC for acute and chronic risks are 0.5 and 1.0, respectively, and for plants, the LOC is 1.0; the LOCs are meant to be protective of community-level effects. The LOCs for aquatic and terrestrial species are summarized in their respective sections below.

4.2.1 Aquatic Exposure Assessment

The labels for diphacinone formulations do not allow applications to water resources. The Diphacinone-50 Conservation label allows for ground and aerial broadcast applications, with aerial applications allowed only when wind speeds are below 35 mph. Diphacinone is slightly mobile in soil and has a low water solubility and low potential for migration to groundwater via leaching or surface water via runoff, indicating a localized effect. The USEPA (2015e) found the exposure of surface water or groundwater to diphacinone is negligible based on the application amounts and methods and the environmental fate properties of diphacinone.

USEPA's (USEPA 2020b) aquatic exposure assessment for diphacinone broadcast applications relied on its estimate for chlorophacinone because USEPA found its estimate for chlorophacinone was inclusive and protective of the potential risks to aquatic organisms from diphacinone; chlorophacinone is more toxic to the test organisms rainbow trout and *Daphnia magna* than is diphacinone. USEPA used the highest modeled exposure in their estimate, which was the broadcast application of chlorophacinone in an orchard. USEPA's analysis included chronic exposure to freshwater fish and *Daphnia magna*, acute exposure to green algae, and acute and chronic exposure to saltwater fish and Mysid shrimp (USEPA 2020b). USEPA found aquatic taxa are not at risk (USEPA 2020b). WS finds its use patterns would also result in negligible exposure to water resources, based on label requirements, diphacinone's environmental fate properties, WS use patterns, and USEPA exposure estimates.

4.2.2 Terrestrial Exposure Assessment

Between 1986 and 2019, there were 122 wildlife incident reports of diphacinone exposure in the Incident Data System maintained by USEPA. Of these incidents, 24 had detectable diphacinone residue in live animals, 29 were highly probable, 15 as probable, and 54 as possible diphacinone exposure (USEPA 2020b). Incidents involving avian species exposure to diphacinone included American kestrel, barn owl, Canada goose, rock dove, wild turkey, and turkey vulture. Mammal

species involved in these incidents included badger, coyote, dog, squirrel, fox, kangaroo rat, opossum, fisher, pig, rabbit, raccoon, skunk, and deer (USEPA 2020b). Federally listed and protected species included bald eagles (2 incidents) and kangaroo rats (1 incident). The suspected exposure scenario was not provided with the incident reports; it is not possible to determine those incidents that resulted from exposure through broadcast applications, belowground applications, or another scenario. These exposures were not from WS applications of diphacinone baits.

Primary Exposure

Direct exposure of nontarget species to diphacinone from belowground applications and belowground caching is mainly limited to animals that can access the burrow. Diphacinone bait may dislodge from the burrow and become accessible to nontarget species aboveground as animals enter and exit the burrow (USEPA 2009), though this would be very minimal. WS expects small mammals that use a treated burrow or colonize it after the target animal has died are likely to ingest a toxic amount of diphacinone bait if it is still present and palatable.

Tamper-resistant bait boxes/stations are required for aboveground applications accessible to the public. They are also used in areas open to hoofed livestock, raccoons, bears, or potentially destructive animals. The use of tamper-resistant bait stations in aboveground applications reduces primary exposure to nontarget species; however, some researchers speculate the constant bait supply may result in the target animal consuming more bait than other application methods, allowing for a higher concentration of diphacinone within the target rodent, which would be a concern for secondary exposure (as summarized in (Baldwin et al. 2021)). However, this was not observed in a study on California ground squirrels, suggesting no increase in diphacinone residues when using bait stations (Baldwin et al. 2021). The authors observed the ground squirrels removed a substantial amount of bait from the bait stations and suggested that the squirrels may have gathered and stored the grain underground for consumption later.

Exposure of nontarget species occurs with aboveground broadcast applications. In a field study to test the efficacy of 0.005% and 0.01% diphacinone bait applied through broadcast or spot application to control California ground squirrels on rangeland, the researchers conducted carcass surveys on study plots to determine the nontarget poisoning rate (Salmon et al. 2007). In the study, bait was applied twice, three days apart, and the search for carcasses occurred for at least 10 days after the final bait application. Sixteen nontarget animals (14 kangaroo rats and 2 deer mice) died from anticoagulant poisoning, representing a nontarget poisoning rate of 0.37 carcasses/hectare. No bird carcasses were collected.

In Washington state, orchards treated with an average of 12.9 kg/ha (11.5 lb/A) of 0.005% diphacinone bait mechanically broadcast to control voles were evaluated for exposure to game birds (Hegdal 1985 unpublished study cited in (USEPA 2004)) (Eisemann and Swift 2006). Most orchards were treated twice, with 20 to 30 days between treatments. The researchers monitored the fate of ring-necked pheasants, California quail, and chukar with telemetry. They collected dead game birds, some of which were shot by hunters. Diphacinone residues were detected in the liver of 10 of 44 ring-necked pheasants (average concentration of 0.23 ppm), 11 of 19 California quail (average concentration of 0.21 ppm), and 1 of 10 chukars. Five pheasants and 10 quail contained bait pellets in the crop. Although exposure to game birds occurred in treated orchards and the birds ate the bait, the risk appeared to be low. During the study, adjoining landowners applied bait at the same time, which may have increased the birds' exposure to treated pellets. Also, the researchers used a combination of wild and pen-raised birds, and the

pen-raised birds may have been more accustomed to foraging on a pelleted diet. The study did not look at long-term effects, such as susceptibility to predation.

Exposure of terrestrial and soil-dwelling invertebrates could occur should they come into direct contact with diphacinone bait aboveground or in burrows. Belowground applications would not result in widespread exposure of invertebrates. Information on the toxicity of diphacinone to invertebrates is limited. In one study, diphacinone residues were quantified in snails and slugs fed baits containing diphacinone for 7 days in the laboratory or collected on or near diphacinone baits in the field (Johnston et al. 2005). Although diphacinone residues were detected in laboratory and field-collected glass snails, and great gray and marsh slugs, no mortality occurred, indicating acute toxicity of diphacinone to snails and slugs is minimal. In another field study, diphacinone residues in target and non-target species were measured pre- and post-application of diphacinone bait hand broadcasted at a rate of 12.82 kg/ha, about 1 kg/ha under the maximum bait application rate on the Diphacinone-50 label (Shiels, 2017). No diphacinone was detected in species sampled pre-application. However, detectable levels of diphacinone were found in non-native mammals (residues of 0.3-32.2 ppm in target rodent species), birds (0-1.2 ppm), gastropods (0-9.8 ppm), and arthropods (0-0.4 ppm) with only one non-target mortality occurring in a single mongoose (diphacinone residue of 0.809 ppm). Skinks did not have detectable diphacinone levels, although the sample size was small (*n*=3).

USEPA (2004) estimated the amount of diphacinone mammals and birds of different weight sizes would need to ingest to receive an LD_{50} dose. A 25-g, 100-g, and 1000-g mammal would need to ingest 1.2 g, 4.6 g, and 46 g, respectively, of diphacinone at a rate of 50 mg a.i./kg-bait to receive an LD_{50} dose. For mammals, the acute LOC was exceeded for all three weight classes after a single day of exposure (50 mg ai/kg-diphacinone bait) and six consecutive days of exposure (USEPA 2020b).

Birds appear to be less sensitive to diphacinone than mammals. At a rate of 50 mg a.i./kg-diphacinone bait, a 25-g passerine would need to eat 200 g of bait (1,000 pellets), a 100-g non-passerine would need to eat 800 g of bait (4,000 pellets), and a 100-g non-passerine would need to eat 8,000 g of bait (>1,000 pellets) to reach an LD_{50} dose. Passerine birds did not exceed the LOC for single-day primary exposure or six consecutive days of primary bait exposure (50 mg ai/kg-diphacinone bait) (USEPA 2020b). Birds that are mainly herbivorous or insectivorous are not expected to be at risk from primary exposure to diphacinone bait. However, USEPA (USEPA 2020b) found a potential chronic risk from diphacinone secondary exposure in birds; USEPA used the avian reproductive data for chlorophacinone to characterize diphacinone's chronic risk to birds.

Exposure of birds to underground applications of bait is expected to be minimal. Exposure of nontarget species during spot bait applications or bait station applications of baits containing 0.01% w/w and 0.005% w/w diphacinone to control ground squirrels in rangeland did not result in take of nontarget birds on treatment plots, but 30 small mammal carcasses were found (Baroch 1996). This represents a nontarget mammal poisoning rate of 0.5 carcasses/ha. However, this may be an underestimate since carcass searches occurred once each day during the baiting period and not beyond the last day of the baiting period. Predators were observed in the study area, including turkey vultures consuming squirrel carcasses, but no secondary poisoning cases were recorded.

Exposure of terrestrial plants is negligible with belowground and aboveground applications in bait boxes.

Secondary Exposure

Secondary exposure could occur for predators and scavengers that can access burrows with poisoned animals or if they kill or scavenge poisoned animals aboveground. Generally, more than one feeding is needed to receive a toxic dose for both the prey animal and predator or scavenger. It can take four to ten or more days for the animal to die after ingesting a toxic dose of diphacinone bait (USEPA 2015e;2020b), and during this time, the animal continues to feed, potentially ingesting a dose well above the toxic dose. Animals that have ingested diphacinone may exhibit behavior (e.g., lethargy) that makes them more susceptible to predators (Cox and Smith 1992 cited in (USEPA 2015e, Vyas et al. 2017)).

The diphacinone residue level in an animal depends on the amount of bait the animal eats, how close together its feedings are, and its metabolism of diphacinone. Diphacinone is metabolized in and disposed of from an animal's body, which may reduce the toxicity to a predator or scavenger. In a laboratory study on the metabolism and disposition of diphacinone, rats were given a single oral dose of radiolabeled diphacinone at 0.2 or 1.5 mg/kg-bw, and mice were given a single oral dose at 0.6 mg/kg-bw (Yu et al. 1982). In rats, about 70% of the oral dose was eliminated in feces and 10% in urine within 8 days. In mice, most of the diphacinone was eliminated within 4 days; only 7% was retained in body tissues. USEPA (2004) summarized an unpublished report by Diaz and Whitacre (1976) in which they orally dosed rats with diphacinone (0.32 mg/kg-bw/day) for 1 or 2 days. In the rats dosed for two days and sacrificed 72 hours after the second dose, about 45% of the diphacinone was excreted (86% in feces, 14% in urine), 25% remained in the body tissues, and 30% was not recovered. In rats dosed once and sacrificed 48 hours later, about 5% of the dose was excreted, 61% retained, and the remaining not recovered. Diphacinone has a short half-life in ground squirrels (67–120 hours in the liver) (as cited in (Baldwin et al. 2021).

USEPA (2004) summarized field studies that evaluated diphacinone residue levels in ground squirrels. The number of days the squirrels were exposed to bait was unknown. Whole carcass residue in squirrels exposed to 50–100 mg a.i./kg-bait had 0.9–1.4 ppm diphacinone whole-carcass residue.

In a study on California ground squirrels, there were no statistically significant differences in diphacinone residue levels in the livers regardless of broadcast, spot treatment, or bait station application method, despite less bait applied by broadcast application (Baldwin et al. 2021). In the study, a 0.01% w/w diphacinone bait was used for broadcast applications, not the 0.005% w/w diphacinone bait used for spot treatments and bait stations (Baldwin et al. 2021). The authors suggest this higher concentration of diphacinone was the reason for the observed (but not significantly different) high residues despite less bait used in the broadcast applications compared to spot treatment and bait station applications. The researchers noted a general trend toward lower residue concentrations when using lower bait concentrations. The formulations WS uses are all 0.005% w/w diphacinone.

USEPA (2004) summarized diphacinone studies looking at toxicity to mammalian predators and scavengers, including mink, mongoose, short-tailed weasel, and dogs. The mammals were given rodents that fed on 0.01% w/w diphacinone bait for 10 days, except for the mongoose that were given rodents that fed on 0.005% w/w diphacinone bait for 5 days. All 3 mink and dogs, 7 of 8 mongoose, and 1 of 2 ermine died.

In the laboratory, secondary exposure of predator and scavenger birds to diphacinone resulted in the death of some exposed birds, and some survivors displayed signs of diphacinone toxicity. Mendenhall and Pank ((1980) cited in (USEPA 2004)) exposed two barn owls for 10 days to

carcasses of rats fed 50 ppm (0.005% w/w) diphacinone bait; the owls did not show signs of toxicity. In this study, the diphacinone residue levels in the rats were not provided. Great horned owls and northern saw-whet owls (*Aegolius acadicus*) given two mice per day that fed on 0.01% w/w diphacinone bait for 10 days caused death in 2 of 3 great horned owls and 1 saw-whet owl (only one bird was exposed) (Mendenhall and Pank 1980, USEPA 2004;2015e). One great horned owl survived but had an increase in blood coagulation time. No American crows or golden eagles given rats fed 0.005% w/w diphacinone bait or meat laced with 2.7 mg/kg-diet, respectively, died from exposure (USEPA 2004;2015e). However, 5 of 11 crows and all eagles showed signs of external bleeding and increased blood coagulation time (USEPA 2004;2015e). USEPA (2015e) summarized that nine percent mortality occurred across these secondary bird exposure studies, which involved 5 bird species and 34 individual birds.

Researchers have observed predators and scavengers hunting anticoagulant-treated areas more than nearby untreated areas (Vyas et al. 2012, Vyas et al. 2017), likely because poisoned target animals become lethargic prior to death and easier to capture (USEPA 2020b). One field study involving chlorophacinone, not diphacinone, demonstrated this. In the field study, Vyas et al. (2017) compared the number of visits by ferruginous hawks to black-tailed prairie dog colonies that were treated with Rozol Prairie Dog Bait (0.005% w/w chlorophacinone, another first-generation anticoagulant that is more toxic to birds than diphacinone (USEPA 2020b) or remained untreated. They found the hawks hunted in only the treated colonies, likely because the poisoned prairie dogs were lethargic and not as aware of their surroundings, making them easier to capture.

Target animals treated with belowground applications usually die belowground; however, a percentage die aboveground. Field studies indicate that 82%–91% of ground squirrel mortalities from diphacinone exposure occur within burrow systems (Baldwin et al. 2021). In a field study across 10 treated prairie dog towns treated with chlorophacinone, not diphacinone, bait, 10 animal carcasses, nine of which were prairie dogs, were found aboveground (USEPA 2009) summarizes (Lee and Hyngstrom 2007). Eight of the carcasses were intact, but two had been scavenged. The rate of prairie dog carcasses was 1 per 14 acres searched. The researchers also noted they saw 5 impaired prairie dogs 10 days or more after bait applications.

Birds that feed on insects, snails, and slugs that have been exposed to diphacinone bait could potentially receive a toxic dose themselves. Johnston et al. (2005) summarized several studies indicating a link between fatalities in birds with the consumption of rodenticide baits (in this case, brodifacoum). Johnston et al. (2005) analyzed diphacinone residues in snails and slugs exposed to bait in the laboratory as well as in the field. As expected, diphacinone residues in snails and slugs that fed on 0.005% w/w diphacinone bait for 7 days in the laboratory were higher than residues in field-exposed snails and slugs, likely due to the variety of food sources available in the field. Mean residue concentrations in glass snail species for laboratory-exposed was 1.77 ppm and field-exposed was 0.69 ppm. Mean residue concentrations in marsh slugs was 2.64 ppm in laboratory-exposed and 0.23 ppm for field-exposed. An estimate of the exposure potential of birds to diphacinone in baiting areas via consumption of gastropods containing diphacinone residues (based on the upper 95% confidence limit for laboratory-exposed gastropod diphacinone residues or 4.93 ppm diphacinone) had risk quotients that were less than the 0.5 level of concern, indicating the mortality risks to nonthreatened or nonendangered birds consuming gastropods containing diphacinone appear to be acceptable (Johnston et al. 2005). However, a conservative estimate exceeded the level of concern for threatened and endangered birds. The authors indicated several assumptions might overestimate this risk, including birds only consumed gastropods with diphacinone residues and residue levels in gastropods in the field being the same as gastropods that were fed a strict diphacinone bait diet in the laboratory.

5 RISK CHARACTERIZATION

This section discusses the quantitative and qualitative risks associated with the proposed use of diphacinone. The evaluation of documented diphacinone health exposure data and relevant animal exposure studies applied to exposure assumption scenarios can quantify the risk of impact to human health and nontarget fish and wildlife if accidentally exposed. Deterministic methods are used, where appropriate, to determine if expected environmental residues exceed toxicity data suggesting possible risk. In other cases, a more qualitative discussion regarding risk may rely on literature and additional information to further elaborate on the potential for injury or harm.

5.1 Human Health

Risks associated with adverse human health are characterized quantitatively for this section's potential accidental occupational exposure scenario. Due to the limited volume applied annually and WS's adherence to label requirements, diphacinone applications to control rodent populations are expected to pose minimal risks to the public.

To quantify the human health risks associated with an accidental occupational dermal exposure to diphacinone during WS pest-control activities, a hazard quotient (HQ) was calculated using the following USEPA risk estimation equation for non-carcinogens:

HQ = Exposure (Dose) / Reference Dose

A hazard quotient of 1 was used to calculate the number of acres a certified applicator could apply 0.005% w/w diphacinone bait. The label-restricted use of diphacinone rodenticides combined with the certified applicator PPE dictates the number of acres a person could work daily without incurring acute or chronic toxicity. The calculated acres a WS employee could safely work per day is summarized in Table 7. The reference dose of 0.00025 mg/kg-bw/day was based on a NOAEL of 0.025 mg/kg-bw/day established from a developmental study which resulted in fetal resorption in pregnant rats after 9 days of exposure to 0, 0.010, 0.025, or 0.075 mg/kg-bw/day diphacinone (gestational days 6–15). Adverse effects were observed in all treatment groups (USEPA 1998). A total uncertainty factor of 100, i.e., factors of 10, $\sqrt{10}$, and $\sqrt{10}$, was applied to the NOAEL to account for genetic variability between humans and variability between animal and human species exposure-response. This observed critical effect is a sub-lethal manifestation of the well-established anticoagulant activity of first-generation anticoagulant rodenticides such as diphacinone. Detailed calculations are provided in Appendix A, Table 1.

WS uses first-generation anticoagulants in addition to diphacinone in its program to control rodents. The effects and environmental impacts of diphacinone in combination with other chemicals, such as pesticides, are poorly understood. As a result, WS personnel could potentially be exposed to multiple compounds of similar metabolic and environmental fate. However, when applied according to label requirements, acute and cumulative exposure risks should be minimal for workers. WS personnel strictly follow product label use requirements, including the proper use of PPE when loading, administering, or handling products containing diphacinone. The risk of public exposure to diphacinone should also be low for the restricted-use formulations (Diphacinone-50 Conservation and the Hawaii SLN). It should not be applied in areas where public exposure could occur. Public exposure would be greater for the general-use formulations that the public can get over the counter. WS applies the general use formulations with the public in mind to minimize potential exposure to the public and uses bait stations where required. Diphacinone baits applied in bait stations that are not consumed by target species would be

expected to be retrieved from the field by WS applicators. Any unrecoverable baits left in the environment from WS use will begin to biodegrade within 45 days.

Table 7. Maximum number of acres a WS employee could safely treat with diphacinone baits in the field per day given different parameters.

Personal protective equipment	Low-concentration ground broadcast (12.5 lb./acre)	High-concentration ground broadcast (20 lb./acre)	†Low application density-Low treatment (7/acre; 4 oz. per application)	†Low application density-High treatment (7/acre; 16 oz. per application)	†High application density-Low treatment (194/acre; 4 oz. per application)	†High application density-High treatment (194/acre; 16 oz. per application)
No gloves/ broken gloves	3	2	19	5	<1	<1
Single-layer PPE	26	19	184	46	7	1.5
Double layer PPE	36	22	255	64	9.2	2

†Application density and treatment levels are based on label restrictions.

Although these health protective recommendations are derived from no observed adverse effect exposures, the critical reference study did not determine a "safe" level of in-utero exposure to diphacinone. As a result, pregnant WS applicators should not work with or around diphacinone and avoid areas treated with diphacinone.

5.2 Ecological Risks

Risk characterization combines information from the dose-response assessment with the exposure assessment to determine the potential adverse effects on aquatic and terrestrial species. In this risk assessment, WS uses USEPA's risk evaluations (USEPA 2011;2020b), peer-reviewed scientific literature, product labels, and WS use patterns and take data (Tables 2 and 3 a, b) to characterize the risks associated with WS applications of diphacinone bait.

5.2.1 Aquatic

The exposure assessment (section 4.2) found aquatic exposure negligible based on WS use pattern, label restrictions, and findings from USEPA's ecological risk assessment (USEPA 2020b). Eisemann and Swift (2006) calculated risk quotients for the aerial application of diphacinone into Hanawi Stream, Hawaii. They found the risk quotients (the comparison of diphacinone's predicted concentration in the water column to the toxicity of diphacinone to aquatic organisms) for fish, crustaceans, or other aquatic invertebrates did not exceed the level of concern. No risk was predicted for any aquatic organism.

Based on the negligible aquatic exposure, label restrictions, and WS use pattern, APHIS considers the risk to aquatic species as negligible.

5.2.2 Terrestrial

The exposure assessment (section 4.2) found that WS applications of diphacinone would result in primary and secondary dietary exposure to terrestrial vertebrates and invertebrates. It can take several days of consecutive feedings to deliver a lethal dose of diphacinone to the target animal and nontarget species (USEPA 2020b). Similarly, secondary exposure to nontarget species would require eating multiple prey items with diphacinone residues over a time period relevant to the metabolic half-life of diphacinone to receive a lethal dose of diphacinone. Additionally, diphacinone residues may contribute to the secondary toxicity of other first-generation and second-generation anticoagulant active ingredient residues in the prey items consumed by nontarget species (USEPA 2020b), but only in areas where additional anticoagulant rodenticides may be in use by non-WS applicators.

WS makes belowground applications directly to burrows to control pocket gophers. Belowground applications reduce but do not eliminate the primary exposure of nontarget terrestrial species. WS monitors burrows for pocket gopher activity, generally fresh mounds, and gophers being very territorial keep other species out of their tunnel systems, which thereby reduces nontarget species primary exposure. WS recognizes nontarget species, mostly small mammals and snakes, would still likely access treated burrows, especially following treatment after pocket gophers succumb to baiting and could be directly exposed to diphacinone bait. WS expects mammals that access burrows and eat diphacinone bait could receive a lethal dose of diphacinone if sufficient amounts of palatable bait remain to allow multiple feedings. Snakes that access burrows are not expected to receive a lethal dose of diphacinone since they will not consume baits but could secondarily by consuming treated pocket gophers. Although some birds may access burrows (e.g., burrowing owls (Vaughan 1961)), WS does not anticipate that burrowing owls will access pocket gopher burrows because they are smaller in diameter than necessary for the owls.

Studies indicate that most target animals that live belowground will die belowground (as cited in (Primus et al. 2001, USEPA 2009) summarizes (Lee and Hyngstrom 2007)), reducing secondary exposure. APHIS expects most of the target animals that WS baits belowground with diphacinone will die underground, reducing exposure potential to nontarget species; however, some will die aboveground. In a study on a different anticoagulant (brodifacoum), 90% of plains pocket gophers died belowground (as cited in (Primus et al. 2001)). Baldwin et al. (2021) found a vast majority of California ground squirrels died belowground (82-91%) following spot treatments, broadcast applications, and bait station applications of diphacinone in rangelands.

Between FY11–20, WS primarily used diphacinone bait to control commensal rodents (Table 2a). However, WS past and expected future use patterns include aboveground spot, mechanical spreader, and aerial applications of Diphacinone-50 Conservation for island conservation projects. During these types of applications, APHIS expects that aboveground treatments of diphacinone bait will result in sublethal effects on birds that consume bait or prey or scavenge on animals with diphacinone residues. Therefore, APHIS expects mortality in birds to be minimal, based on their relatively low sensitivity to diphacinone, the requirement for multiple feedings for lethality, and their foraging preferences (they are not attracted to the bait, their diet is diverse, and so on), as well as, with the exception of some island conservation applications, their foraging area is often larger than the treatment area. USEPA (2004) conducted a comparative risk evaluation of several rodenticides and concluded the primary risks of diphacinone to birds were low, and secondary risks were moderate. Using birds as surrogate species, consuming diphacinone bait by terrestrial amphibians would have the same primary and secondary risks as birds. Snakes are sensitive to diphacinone but are unlikely to be attracted to the diphacinone baits themselves, so they must be exposed to diphacinone residues over multiple feedings on exposed prey to have

secondary risks. Aboveground spot, mechanical spreader, and aerial applications would impact nontarget mammals through primary and secondary exposure; USEPA (2004) estimated mammalian primary and secondary risks to diphacinone were high.

Insects that feed on bait may subsequently be consumed by terrestrial vertebrates; however, the terrestrial vertebrates are not likely to ingest a toxic dose of diphacinone given the amount of diphacinone carried inside the insect, and the number of poisoned insects the terrestrial vertebrates would need to eat within a toxicity-relevant timespan to receive a lethal dose. Johnston et al. (2005) estimated risks to juvenile and adult bobwhites and mallards consuming snails and slugs with diphacinone residues in Hawaii. In their estimate, they assumed the birds solely ate gastropods containing 4.93 ppm diphacinone (the highest 95% percentile residue concentration in gastropods fed only 0.005% w/w diphacinone bait in the laboratory). They concluded the broadcast application of diphacinone baits in Hawaii would have an acceptable level of risk for non-threatened birds. However, their estimate did find risks to endangered bird species consuming gastropods with diphacinone residues, indicating the use of diphacinone bait in the range of threatened or endangered species should be avoided in most circumstances. In some situations, diphacinone baits targeting invasive rodents or mongooses are used to benefit threatened or endangered species in a conservation area or on islands.

WS has no records of nontarget take through its use of diphacinone baits. However, APHIS recognizes it is impossible to detect all nontarget take because animals may die belowground, be difficult to find in certain habitats, or are wide-ranging and dispersed outside of the treatment area. WS does monitor proposed treatment sites and does not treat areas with threatened and endangered species outside of conservation uses.

The Diphacinone-50 Conservation and Ramik® Mini Bars All-Weather Bait labels provide instructions to collect and dispose of dead, exposed animals during and after treatment but do not provide the time interval after treatment for doing this. The Ramik® Mini Bars All-Weather Bait labels also instruct applicators to remove unused bait. Similarly, the Eaton's Gopher Bait Block label and Bait Block® Rodenticide with Peanut Butter Flavorizer™ (and Apple Bait Block® Rodenticide) require applicators to remove dead animals and unused bait; the Eaton's Gopher Bait Block label indicates to check the treatment area for 10 to 15 days after treatment. Despite these mitigations, poisoned animals that are aboveground between searches or are missed during searches remain available to predators and scavengers.

APHIS expects minimal risk to terrestrial plants based on lack of exposure through WS use pattern and diphacinone's mode of action. The reduction in available prey in an area from diphacinone treatment could impact food availability for predators; however, APHIS anticipates predators would extend their hunting range into new areas. This document uses the risk assessments for diphacinone (USEPA 2004;2020b), as well as assessments published in the scientific literature (e.g., (Eisemann and Swift 2006)) to assist in estimating risks associated with WS use patterns; these risk assessments are conservative and assume nontarget species feed solely on diphacinone bait or only on animals with diphacinone residues. Although this assumption is unlikely to occur in nature, APHIS still finds WS use of diphacinone could negatively impact terrestrial mammals that ingest bait or feed on animals that contain diphacinone residues. To reduce the exposure potential to nontarget animals, WS complies with label instructions to reduce nontarget animal exposure (e.g., removal of unused bait and carcasses), monitors treatment areas for nontarget species activity, and evaluates each treatment site and selects a label-approved application method that would minimize exposure potential.

6 UNCERTAINTIES AND CUMULATIVE IMPACTS

The uncertainties associated with this risk evaluation arise primarily from the limited toxicity information available for the various diphacinone FGAR formulations. Unpublished acute toxicology studies submitted to USEPA by registrants and individual formulation safety data sheets provide limited acute mammalian toxicity values, which can be used to make general conclusions concerning the impact of diphacinone on humans and the non-target environment.

Other uncertainties related to chronic and sublethal effects data for some fish and wildlife and surrogacy of test organisms are typical for most pesticides; however, there is a considerable amount of field data related to poisoning of nontarget wildlife with anticoagulant rodenticides. This information provides a weight-of-evidence approach to conservatively evaluate the risk of diphacinone to nontarget organisms. The conservative assumptions regarding the potential for exposure to human health, nontarget species, and the environment address the uncertainties to some extent. A lack of risk using these conservative assumptions supports the reasonable certainty that impacts on human health and the environment will be negligible. However, primary and secondary risks to nontarget mammals are not negligible, particularly for broadcast applications.

Diphacinone use patterns vary for different sites and application methods. This risk assessment used conservative assumptions regarding human exposure to account for variations in use patterns and ensure that risks are not underestimated for workers. Individuals with impaired liver function may be at greater risk than other population members because first-generation anticoagulants such as diphacinone inhibit the synthesis of vitamin K and are metabolized by liver mixed-function oxidases (i.e., the cytochrome P450 enzyme system).

Another area of potential uncertainty in this risk assessment is the potential for cumulative impacts on human health, nontarget species, and the environment from the proposed use of diphacinone in the WS program. Areas where cumulative impacts could occur are: 1) repeated worker and environmental exposures to diphacinone from program application; 2) co-exposure to other chemicals with a similar mode of action; and 3) exposures to other chemicals affecting the toxicity of diphacinone.

Repeated exposures that could lead to increased risks of injury from accidental diphacinone exposure by WS applications are expected to be minimal due to strict WS applicator adherence to label-required PPE. To minimize the risk of cumulative impacts of repeated exposures, WS applicators could consider a pesticide application rotation schedule that rolls applicators off and on application assignments weekly. Diphacinone baits can only be administered using tamper-resistant applications in areas where people reside or can access the product. These measures reduce the potential for cumulative impacts related to exposures to diphacinone and other stressors.

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8 PREPARERS: WRITERS, EDITORS, AND REVIEWERS

8.1 APHIS WS Methods Risk Assessment Committee

Writers for "Use of Diphacinone in Wildlife Damage Management Risk Assessment":

Primary Writer: Andrea Lemay
Position: USDA-APHIS-Policy and Program Development (PPD), Environmental and Risk Analysis Services (ERAS), Biological Scientist, Raleigh, NC
Education: BS Plant and Soil Science (Biotechnology) - University of Massachusetts; MS Plant Pathology - North Carolina State University
Experience: Thirteen years of service in APHIS conducting risk analysis. Nine years of experience in preparing environmental analyses in compliance with the National Environmental Policy Act.

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Education: B.S. Environmental Science – University of Florida; MPH Industrial Hygiene-University of South Carolina, Ph.D. Toxicology-Florida Agriculture and Mechanical University
Experience: Ten years of experience conducting human toxicological research at Florida Agriculture and Mechanical University, University of Nebraska Medical Center, and Tulane University. Four years of experience conducting human health and environmental toxicological risk assessments and assisting environmental compliance programs at the Florida Department of Health, Commonwealth of Pennsylvania, and USDA.

Writer: Thomas C. Hall
Position: USDA-APHIS-WS, Operational Support Staff, Staff Wildlife Biologist, Fort Collins, CO
Education: BS Biology (Natural History) and BA Psychology – Fort Lewis College; MS Wildlife Ecology – Oklahoma State University
Experience: Special expertise in wildlife biology, identification, ecology, and damage management. Thirty-seven years of service in APHIS Wildlife Services including operations and research in CO for research and OR, GU, CA, OK, and NV for operations conducting a wide variety of programs including bird damage research and management, livestock protection, invasive species management, wildlife hazard management at airports, property and natural resource protection including waterfowl, brown tree snake, feral swine, rodent, and beaver damage management. Applied and supervised chlorophacinone use.

Writer: Jim Warren
Position: USDA-APHIS-Policy and Program Development (PPD), Environmental and Risk Analysis Services (ERAS), Environmental Toxicologist, Little Rock, AR
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Editors/Contributors for "Use of Diphacinone in Wildlife Damage Management Risk Assessment":

Editor: Shelagh DeLiberto
Position: USDA-APHIS-Wildlife Services (WS), Environmental Coordinator, Fort Collins, CO
Education: BA Biology and Environmental Science – Ithaca College; MS Wildlife Biology – Colorado State University
Experience: Sixteen years of service in APHIS conducting wildlife research. Two years of experience in preparing categorical exclusions and environmental analyses in compliance with the National Environmental Policy Act.

Editor: Emily Ruell
Position: USDA-APHIS-WS, NWRC, Registration Manager, Fort Collins, CO
Education: B.S. Zoology and Biological Aspects of Conservation – University of Wisconsin - Madison; M.S. Ecology – Colorado State University (CSU); M.A. Political Science – CSU
Experience: Nine years of experience with APHIS WS NWRC preparing and reviewing vertebrate pesticide registration data submissions and other registration materials, and providing pesticide regulatory guidance to WS, WS NWRC, and collaborators. Prior experience before joining APHIS includes seven years of conducting field and laboratory wildlife research at CSU, and environmental policy research for the U.S. Geological Survey.

8.2 Internal Reviewers

USDA APHIS Wildlife Services

Reviewer: Gary Witmer
Position: Supervisory Wildlife Biologist and Research Project Leader (Retired)
Education: BS and MS in Biology, University of Michigan; MS in Wildlife Ecology, Purdue University; Ph.D. in Wildlife Science with minors in Statistics and Forest Management, Oregon State University.
Experience: Thirty years researching wildlife populations, damage, and damage management with an emphasis on rodents and about 300 scientific publications.

Reviewer: Aaron Shiels
Position: USDA-APHIS-WS, National Wildlife Research Center: Research Biologist and Rodents Project Leader, Fort Collins, CO
Education: BS Environmental Sciences, University of Denver; MS Biology, University of Nevada, PhD Botany/Ecology University of Hawaii
Experience: Fifteen years researching rodent damage management techniques including the use of rodenticides.

Reviewer: Tyler Bogardus
Position: USDA-APHIS-WS, Hawaii Operations, District Supervisor, Kapolei, HI.
Education: BS Wildlife Biology – Colorado State University
Experience: 15 years conducting predator control and management specializing in small vertebrates.

Reviewer: Jon Grant
Position: USDA-APHIS-WS, State Director, Albuquerque, NM

Education: BS Wildlife - Univ. Wisconsin, Stevens Point
Experience: Expertise in wildlife biology and wildlife damage management operations and research. Thirty years of service in APHIS Wildlife Services operational programs in WI and NM. Experience in a wide variety of damage management to include feral swine, livestock protection, bird damage, wildlife hazard management at airports. Have extensive experience applying and supervising the use of zinc phosphide.

Reviewer: Ken Gruver
Position: USDA-APHIS-WS AL/PR/USVI State Director
Education: BS Wildlife Management – Sul Ross State University, MS Wildlife Management – Sul Ross State University
Experience: Thirty-two years service with APHIS WS conducting and supervising Wildlife Damage Management Research and Operations

Reviewer: Donald J. Wilda
Position: USDA-APHIS-WS, State Director MA, CT & RI, Amherst, MA
Education: BS Wildlife Biology-University of Massachusetts
Experience: Thirty-one years of service in APHIS conducting and supervising wildlife damage management

8.3 Peer Review

The Office of Management and Budget requires agencies to have peer review guidelines for scientific documents. The APHIS guidelines were followed to have "Use of Diphacinone in Wildlife Damage Management" peer reviewed. WS worked with the Association of Fish and Wildlife Agencies to have experts review the documents.

8.3.1 Peer Reviewers Selected by the Association of Fish and Wildlife Agencies

Texas Parks and Wildlife Department

Louisiana Department of Wildlife and Fisheries

Arizona Game and Fish Department

8.3.2 Comments

1. The one deficiency is in the description of the ecological effects on birds, although the issue is somewhat addressed in the terrestrial exposure assessment, and risk characterization sections. Given the very wide dose (>100X) range for the species listed and the documented susceptibility of raptors to secondary toxicity from anticoagulant rodenticides, I believe the risk to nontarget species is underestimated. Additionally, some of the target species are more likely to die above ground than others (black rats e.g.). I would recommend an additional discussion of this concern in 5.2.2. While carcass removal should mitigate this if executed diligently, incorporating a metric for carcass removal could enhance this mitigation method.

   Response: We disagree that the risk to nontarget species is underestimated. We acknowledge in Section 5.2.2 that “WS applications of diphacinone would result in primary and secondary dietary exposure to terrestrial vertebrates and invertebrates.” The risk of diphacinone exposure to these species exists regardless of the range of toxicity values reported. WS avoids threatened and endangered species, although some WS

diphacinone applications are to protect sensitive species. WS does not have a metric for carcass removal but may pick up carcasses that are present above ground. While black and brown rats may be more likely to die above ground, WS has not used diphacinone for black rat control in the last 7 years and has used only 16.7 pounds per year for brown rat control.

2. There is no mention on the impact of Diphacinone on avian scavengers such as turkey vultures and black vultures. Based on the studies in owls showing secondary exposure morbidity and mortality, is there concern for vultures being at a higher risk of Diphacinone toxicity?
   Response: Available literature on vulture exposure to diphacinone does not indicate that they are at a higher risk for morbidity or mortality. A study that observed vultures feeding on ground squirrel carcasses after a diphacinone application did not record any secondary poisoning cases (See Section 4.2.2). Several studies of anticoagulant residues in non-target wildlife found few or no turkey or black vultures with diphacinone residues (New York: Stone et al. Anticoagulant rodenticides and raptors: recent findings from New York, 1998-2001. Bulletin of Environmental Contamination and Toxicology 70:34-40, California: Hosea, R.C. 2000. Exposure of non-target wildlife to anticoagulant rodenticides in California. Proceedings of the Vertebrate Pest Conference 19: 236-244, Oregon: Herring et al. 2023. Anticoagulant rodenticides are associated with increased stress and reduced body condition of avian scavengers in the Pacific Northwest. Environmental Pollution 331: 121899).

3. How long does Diphacinone degradation by degradative photolysis take in water at a ph7-9? It was mentioned to have a half life of 44 days at a pH of 5 through hydrolysis but no mention of a timeline for degradative photolysis at ph7-9.
   Response: Diphacinone photolysis is reported as stable at pH 7-9, so there is no timeline for degradation. However, although we discuss in Section 2.2 that diphacinone will undergo degradative photolysis due to sunlight in water, there is no published timeline for this process.

4. Is there concern for secondary effects in aquatic biota? Ex. A moderately toxic load in a fish that is then ingested by an aquatic mammal such as an otter?
   Response: There is negligible exposure of diphacinone to water resources due to WS use patterns and label restrictions preventing applications to water resources (See Section 4.2.1). However, a recent study in Germany (Regnery et al. 2024. First evidence of widespread anticoagulant rodenticide exposure of the Eurasian otter (*Lutra lutra*) in Germany. Science of the Total Environment 907: 167938) studied anticoagulant rodenticide residues in Eurasian otters and found 23% of the 122 otters tested had chlorophacinone residues (the study did not test for diphacinone residues). Previous research has shown that sublethal anticoagulant exposure in birds and mammals may affect their disease susceptibility and immune function (Rattner et al. 2020 Brodifacoum toxicity in American kestrels (*Falco sparverius*) with evidence of increased hazard on

subsequent anticoagulant rodenticide exposure. Environmental Toxicology and Chemistry 39: 468-481).

5. Is there concern for people eating fish that have exhibited any toxic load?
   Response: There are no published studies on the risks of human ingestion of fish exposed to diphacinone. However, a study of brodifacoum residues in coastal marine species showed little risk of secondary poisoning to humans. Brodifacoum residues in blue cod (*Parapercis colias*) were largely in the liver tissue, not in muscle tissue that would be consumed (Masuda et al. 2015. Residue profiles of brodifacoum in coastal marine species following an island rodent eradication. Ecotoxicology and Environmental Safety 113: 1-8). Current use patterns and label restrictions for WS uses of diphacinone suggest that the potential for aquatic exposure is negligible. Although diphacinone exhibits chemical fate properties that suggest it may bioconcentrate the likelihood of exposure that could result in significant residues in fish tissue that result in adverse effects to people is not anticipated. Island conservation uses are evaluated on a case-by-case basis regarding exposures to human populations. In previous island conservation uses where there has been a concern about rodenticide exposure for people through ingestion of aquatic biota there have been fish harvest moratoriums. These may be implemented for island conservation uses where there is a greater potential for aquatic exposure to rodenticide residues in human populations.

6. I didn't see any mention of avoiding or limiting treatments when an area has an active hunting season, especially considering nontarget mortality or detectability in game species. While the risk is perhaps minimal for human ingestion, this is a reasonable expectation to safeguard human health and is a largely avoidable risk. What would be a reasonable withhold period considering bait degradation and clearance time in possibly affected game animal species?
   Response: Wildlife Services adheres to all label requirements. None of the registered diphacinone products that WS uses have hunting season restrictions. We discuss game bird exposure to diphacinone baits broadcast to control voles in Section 4.2.2. Although exposure to game birds occurred in treated orchards, and there was evidence of bait consumption (bait in the gastrointestinal tract), residues ranged from below the method limit of detection (0.1 ppm) to 0.56 ppm diphacinone in liver samples. No diphacinone residue was detected in muscle tissue (Hegdal 1985. Unpublished NWRC report (U02591)) Primary hazards to game birds associated with the use of ramik brown (diphacinone bait) for controlling voles in orchards. Draft Report 188 pp). A study of diphacinone residues in wild pigs exposed to broadcast diphacinone baits resulted in residues of 0.251 ppm in pig muscle and 3.07 ppm in pig liver (Pitt et al. 2005. Diphacinone residues in free-ranging wild pigs following aerial broadcast of rodenticide bait in Hawaiian Forests. Unpublished report, QA-1077, National Wildlife Research Center, Fort Collins, CO. 35 pp). In a pen study with domestic pigs dosed with diphacinone-laced food items, the highest reported residues were 0.37 ppm in pig muscle and 3.22 ppm in pig liver (Fisher 2006. Diphacinone in pigs: sublethal exposure and residual persistence in tissues. Proceedings of the Vertebrate Pest Conference 22:434-439). These are worst-case

scenario residues if a pig happens upon large quantities of bait in one place. Even though muscle and fat are most commonly consumed, Fisher (2006) determined that based on the terminal half-life estimate for pig liver (104 days), a withholding period of 156 days is suggested.

7. If no withhold period, would a likely exposure from consuming contaminated meat have any significant physiological effects for the average person? What about for most vulnerable (i.e pregnancy, liver failure, etc.)
   Response: Eisemann and Swift (2006; Ecological and human health hazards from broadcast application of 0.005% diphacinone rodenticide baits in native Hawaiian ecosystems. Proceedings of the Vertebrate Pest Conference 22: 413-433.) determined that based on maximum residues described in Pitt et al. (2006) of 0.25 mg/kg pig muscle, 3.07 mg/kg pig liver, a 55 kg person would have to consume 28.49 kg of pork meat and 2.33 kg of pork liver per day to reach a dose of diphacinone equivalent to that affecting blood clotting in rats. Pregnant women of the same weight would have to ingest 5.5 kg meat or 0.45 kg liver per day for the amount of diphacinone equivalent to the dose that has been shown to cause fetal reabsorption in rats. Based on these calculations, Eisemann and Swift (2006) determined that the risk to people eating meat from birds and pigs harvested within diphacinone treatment areas is very low. These risk estimates are based on conservative exposure scenarios where wild pigs would have free access to large quantities of broadcast applied bait. This is unlikely to occur since significant bait removal by nontarget species would preclude the use of diphacinone or other rodenticides for rodent eradication and control projects.

Comments received not requiring a response.

1. The quality, methods and completeness of the risk assessment of this WDM are good. The uncertainties are well described including ones that address the concerns detailed in my comments.
2. I find the document to be very thorough and contains many details for the anticoagulant rodenticide diphacinone. Consequences and potential consequences for the use of diphacinone in wildlife damage management are well illuminated in the document.
3. Great detail into mitigating actions for different products were offered, with very concise instructions for the use of the different products and for different animals. These mitigating actions are somewhat common sensical but clear definition in the document ensures proper use of these toxic products.
4. All estimates and assumptions were clearly stated and obvious. There was no hint of subversion or hiding of these actions.
5. The reference list was extensive and subject matter detailed, and seems well vetted. The document is very professional and well supported by the citations.
6. Overall, I think this is a high quality document. Very thorough in content and details the negatives as well as the benefits of use of the diphacinone products. The science is good. The detailed information on human health and safety, even down to outlining how many baits could be safely put out with damaged gloves, was exemplary. A true reference document.

APPENDIX A. Risk Characterization for Accidental Occupational Exposure* to Diphacinone

Parameters	Values	Values	Values	Sources
PPE used (gloves)	**‡No gloves or damaged PPE**	**‡Single layer gloves**	**‡Double layer PPE**	**Reference**
PDR exposure; PDR=UE*AR*A*ABS				
UE applicator granules by hand	104	71	40.3	(USEPA 2021a)[1]
Application	**AR (A/day)**	**AR (A/day)**	**AR (A/day)**	**Calculated From or Reference**
Low Conc. GB 12.5 lb./acre	0.00063 (3)	0.00063 (26)	0.00063 (36)	
High Conc. GB 20 lb./acre	0.001 (2)	0.001 (18.5)	0.001 (22)	
†Low Application Density-Low Treatment (7/acre; 4 oz. per application)	0.00009 (19)	0.00009 (184)	0.00009 (255)	Calculated from (USEPA 2019a)[1]
Low Application Density-High Treatment (7/acre; 16 oz. per application)	0.00035 (5)	0.00035 (46)	0.00035 (64)	Calculated from (USEPA 2021a)[1]
High Application Density-Low Treatment (194/acre; 4 oz. per application)	0.0024 (<1)	0.0024 (6.6)	0.0024 (9.2)	Calculated from (USEPA 2021a)[1]
High Application Density-High Treatment (194/acre; 16 oz. per application)	0.0097 (<1)	0.0097 (1.5)	0.0097 (2)	Calculated from (USEPA 2021a)[1]
ABS	0.1	0.01	0.01	(USEPA 2007)[1]
PDR (mg/day)	0.0175	0.0175	0.0175	Calculated
BW	70	70	70	(USEPA 2007)[1]
Dose (mg/kg-bw/day)	0.00025	0.00025	0.00025	Calculated
RfD (mg/kg-bw/day)	0.00025	0.00025	0.00025	
Hazard Quotient	1	1	1	**

PPE = Personal protective equipment **PDR** = Potential Daily Rate **UE** = Unit Exposure or mg/lb a.i. **AR** = Application Rate (lb a.i./acre) **A** = Acres **GB** = Ground Broadcast **Conc.** = Concentrate **ABS** = Fraction absorbed dermally **BW** = Body weight (kg) **RfD** = Reference Dose

†Application density and treatment levels are based on label restrictions.

‡ Based on hand application estimates of exposure by Pesticide handler exposure database

*Exposure risks are calculated from dermal and inhalation exposures.

** Based on NOAEL of 0.025 mg/kg-bw/day, 9-day developmental study (see Section 3)

[1]Appendix References in Section 7. Literature Cited.