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ABSTRACT: The National Wildlife Research Center (NWRC) conducted a field study to determine hazards to non-target gallinaceous birds following the use of 2.0% zinc phosphide (Zn_3P_2) baits for vole control in fall alfalfa. Consultation among the NWRC, USDA Wildlife Services, California Department of Food and Agriculture (CDFA), U. S. Environmental Protection Agency (EPA), and the California Department of Fish and Game produced a 3-phased study. Free-ranging ring-necked pheasants and California quail were studied in alfalfa during the concurrent harvest of other agricultural crops. These data would be used by CDFA to support the re-registration of their label "Rodent Bait Zinc Phosphide Treated Grain (2.00%)", EPA Reg. No. CA890027. Phase 1 was a pilot study to determine whether the two test species could be maintained in walled enclosures. Phase 2 was a worst-case-scenario using the test species in alfalfa enclosures during vole control (i.e., simulated field study). Information from the 14-day post-baiting period led to a better understanding of some variables, including the sub-lethal effects that could impact the design of the final phase. During Phase 3 the actual non-target field study was conducted. Results from Phase 1 showed that these species could be maintained in outdoor enclosures using only wing clipping, 1 m-high metal walls, and no covering nets. Phase 2 proved that in outdoor alfalfa enclosures, baiting for vole control was not hazardous to quail but might be to pheasants. Phase 3 concluded that 2.0% Zn_3P_2 bait when applied per label directions was not hazardous to either wild or pen-reared free-ranging pheasants in fall agricultural areas. This article summarizes the 3-phased study, the resulting data, and conclusions.

KEY WORDS: alfalfa, non-target hazard, *Phasianus colchicus*, ring-necked pheasant, zinc phosphide

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INTRODUCTION

Chinese ring-necked pheasants (*Phasianus colchicus*) occur throughout the United States, and they are associated with many different terrestrial habitats. The California Department of Fish and Game (CDFG) suggested that some of the highest densities of ring-necked pheasants in the United States occur in agricultural areas in the northern Sacramento Valley (CDFG 1962, Littrell 1990). Free-ranging pheasants in California include both wild and pen-reared birds (CDFG 1962). Formerly CDFG and currently many private pheasant clubs in California release numerous pen-reared pheasants (300,000 - 1,000,000 /yr) for fall hunts (Hart 1990). This study was designed to detect the non-target hazard posed to these upland game birds in mixed crops when zinc phosphide is used in fall alfalfa (*Medicago sativa*) fields for vole control. In June 1993, the California Vertebrate Pest Control Research Advisory Committee (CVPCRAC), through the California Department of Food and Agriculture (CDFA), signed a Cooperative Agreement with the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Wildlife Services, National Wildlife Research Center (NWRC) to conduct this research. CDFG and the Sutter County Department of Agriculture (SCDA) provided additional support.

HISTORY

Zinc phosphide (CAS No. 1314-84-7) (Zn_3P_2) is a relatively broad-spectrum rodenticide, and its mode of action is the release of phosphine gas during hydrolysis in the gastrointestinal tract of poisoned animals. It has a variety of agricultural uses, as enumerated by Hood (1972) and Fagerstone and Ramey (1996), including the control of jackrabbits (Evans et al. 1970) and prairie dogs on rangeland (Tietjen and Matschke 1982), nutria in agricultural areas (Evans et al. 1966), rats in sugarcane (Doty 1945, Hilton et al. 1972, Pank 1976) and macadamia nuts (Fellows et al. 1978), and voles in orchards (Hegdal and Gatz 1977). Secondary poisoning has not been an issue with Zn_3P_2 because it decomposes rapidly in the gastrointestinal tract of poisoned animals (Evans 1967, Savarie 1981, Johnson and Fagerstone 1994). In the environment, Zn_3P_2 breaks down when exposed to wet conditions (Zbirovsky and Myska 1957), and its toxicity decreases (Hayne 1951; Hilton et al. 1972; Ramey et al. 1994b, 2000; Sterner and Ramey 1995). Zinc phosphide was undergoing re-registration by the U. S. Environmental Protection Agency (USEPA) in 1993 under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (FIFRA 1988) as amended in 1988 (USEPA 1988; Ramey et al. 1992, 1994a).

Rodents, particularly voles, can be important agricultural pests in the western U.S. and may cause extensive damage to agricultural crops including alfalfa (Marsh 1988, Lewis and O'Brien 1990). Voles cause an estimated $8.5 million annual loss in California alfalfa production (J. Clark, pers. commun., 1993). CDFA sought efficacy and non-target data for their label titled "Rodent Bait Zinc Phosphide Treated Grain (2.00%)," USEPA Reg. No. CA890027. This bait was manufactured as steam-rolled-oat (SRO) groats and was broadcast to control California voles (*Microtus californicus*) and montane voles (*M. montanus*) in alfalfa. Label application specifications for this bait were 5.6 - 11.2 kg/ha

(5 - 10 lb/ac), to control vole populations in alfalfa following the last fall harvest.

PILOT STUDY – PHASE 1

In Phase 1, 12 pen-reared California quail (*Callipepla californica*) and 12 Chinese ring-necked pheasants were placed in a 0.2-ha (0.5-ac) enclosure. Enclosure walls were constructed of 1-in chicken wire with a height of 1 m and reinforced at 3 m (~10 ft) intervals with metal fence posts. No netting was provided overhead. The study was designed to determine whether wing clipping alone was sufficient to keep these species in these enclosures, and whether clipping 1 wing versus 2 was more efficacious. Six birds of each species had 1 wing clipped and the remainder had both wings clipped. Over a period of 5 days, it was concluded that both species could be retained in the enclosure for a short period of time by clipping both wings for quail (100% remained) and clipping 1 wing for pheasants (100% remained).

ENCLOSURE STUDY – PHASE 2

In Phase 2, two concurrent investigations (A and B) were conducted by the NWRC at Oregon State University's Hyslop Farm in cooperation with staff from the Department of Fisheries and Wildlife. Investigation A determined the efficacy of 2.0% Zn_3P_2 use for vole control in alfalfa. Investigation B studied the non-target hazard this use would pose to pheasants and quail. A USEPA technical paper provided additional suggestions for performing terrestrial field studies to address potential adverse effects that a proposed pesticide use may have to non-target wildlife (Fite et al. 1988). Terrestrial field studies (Level 1) are screening studies, which essentially determine if adverse impacts occur to non-target wildlife under actual pesticide use conditions. An objective of this USEPA field study (GDLN 71-5a [Level 1]) was to examine potential effects of Zn_3P_2 on wildlife from direct poisoning by ingestion, and if possible, to gather information about any sub-lethal toxic effects and/or altered behavior (USEPA 1988).

These two simulated field studies (Investigations A and B) were undertaken concurrently in 0.2-ha (0.5-ac) enclosures planted with mature alfalfa (>35 cm). Investigation A provided efficacy data from the broadcast baiting of 2.0% Zn_3P_2 steam-rolled-oat (SRO) groats for vole control after introducing 23 or 24 voles in all 18 enclosures. Six enclosures contained voles only and 12 enclosures had both 23 or 24 voles and 8 or 9 birds, either pheasants or quail. Greater than 94% mortality of gray-tailed voles (*Microtus canicaudus*) was reported in Investigation A using 2.0% Zn_3P_2 on steam-rolled-oat (SRO) groats (Reg. No. CA8900-27) in alfalfa (Sterner et al. 1994). Investigation B recorded the potential non-target hazard to ring-necked pheasants or California quail involved in the use of 2.0% Zn_3P_2 for vole control during a 14-day post-baiting period. Six enclosures were randomly assigned to each wing-clipped bird species (e.g., 3 enclosures were randomly baited with 0.0% Zn_3P_2 and 3 with 2.0% Zn_3P_2). Bare ground was maintained within 1 m of all enclosure walls to decrease vole use of these areas and lessen the likelihood of escaping. The bait was applied on September 30, 1993 per label directions; however, some of the bait was applied on bare ground. Twenty-four pheasants and 24 quail (~½ of each species) were equipped with radio-transmitters to determine their locations and movements twice daily, morning and late afternoon. Mortality radio-transmitters were used that doubled the pulses per minute (ppm) when the birds remained motionless for >1 hr. The use of these transmitters greatly facilitated the determination of the sub-lethal effects of the bait versus the lethal effects.

The authors designed Investigation B in part as a worst-case scenario for upland birds for several reasons. First, all pen-reared birds (52 pheasants and 51 quail) were wing-clipped as indicated by Phase 1 results, thus negatively impacting their flight behavior and exposing them to 2.0% Zn_3P_2 24 hrs a day for 14 days. Second, normally pheasants and quail are granivorous in the fall and use alfalfa predominantly for cover and not for foraging. Third, the 1 m of bare ground near the walls of each enclosure provided easily observable 2.0% Zn_3P_2 oat groat baits, and the birds used these barren areas more frequently than the alfalfa especially when foraging, sunning, and dusting. Therefore, we expected most if not all of the pheasants and quail would die over the course of the 14-day post-baiting period; however, we might be able to observe the sub-lethal toxic effects in each species. We correctly believed this information would be helpful in designing the actual field study to be completed in Phase 3.

Results from this study showed Zn_3P_2 mortality occurred in 64% (18/28) of the pheasants and none (0/26) of the quail. All Zn_3P_2-related mortality (16 baited and 2 escapees into baited enclosures) occurred within 48 h of baiting; the occurrence of all but one Zn_3P_2 death (17/18) within 24 hrs of exposure to the bait was highly significant ($P < 0.00001$) versus mortality observed pre-baiting and >1 day post-baiting. All Zn_3P_2 deaths were confirmed by necropsy and the number of treated SRO groats eaten by each pheasant was determined. Surviving birds were euthanized at the completion of the study and examined for bait.

Survival ratios between 0.0% Zn_3P_2-baited and 2.0% Zn_3P_2-baited birds were different ($P < 0.01$) for pheasants, indicating the non-target hazard to ring-necked pheasants was significant, but not for quail. Zn_3P_2-poisoned pheasants had an average of 180 (SD ± 93) Zn_3P_2 SRO groats in their crops. Surviving pheasants and quail from 2.0% Zn_3P_2 baited enclosures did not have SRO groats in their crops at 14 days post-baiting. Mortality associated with other factors (predators 6%, accidents and sickness 4%, and escapes 3%) was not significantly different between avian species, baited groups, or radio-collared versus non radio-collared birds. Eight birds (~8%, 8/103) were missing at the completion of the study as some birds had regained flight near the study's completion.

Results of Investigation A (>94% mortality for voles) have been described (Sterner at al. 1994). Likewise, more details about the possible non-target hazard to pheasants have also been presented (Ramey et al. 1994b, Ramey and Sterner 1995) and will be only summarized here. Also, a concurrent study of the bait's weatherability during the enclosure study indicated its toxicity decreased over time; therefore, its non-target exposure and environmental risks to quail and pheasants decreased over the 14-day post-baiting period (~37% over the first 24 hrs). These weatherability results are reported in Ramey et al. (1994b) and Ramey and Sterner (1995) and are similar to findings in other studies (Breyl et al. 1973).

Sub-lethal toxic effects were observed in 2 roosters, but not in 4 surviving hen pheasants. Signs of Zn_3P_2 poisoning just before death were not observed in this study, because all Zn_3P_2 deaths occurred overnight. However, signs of lethal toxicity have been described by Janda and Bosseova (1970) in the laboratory while conducting LD_{50} determinations for partridges (*Perdix* spp.) and pheasants fed 2.5% Zn_3P_2 wheat baits. They observed that the first sign of toxicity was listlessness; the birds hid and soon became incapable of movement, and they could be easily approached by the investigators. Initially, respiration was slow and deep; later, it became quicker and shorter. The latent period of these effects was 2 - 6 hours. Next, lethargy occurred and was followed by stiffness. The birds remained rigid until the moment of death. Most of the birds died within 12 - 24 hours. Similar behavior prior to death from Zn_3P_2 poisoning have been reported by Hudson et al. (1984); they stated pheasants died in undisturbed vegetation rather than in the open and showed no signs of a struggle.

In Investigation B, instrumented hens were observed each day post-baiting, and they were observed running, foraging, and even attempting to fly from the enclosures. In contrast, the morning following baiting (<24 h post-baiting) the 2 roosters hid in heavy cover and seemed to be incapable of movement. Because the mortality mode of the transmitters did not activate, we believed the birds were alive but only moving slightly. Both roosters were easily approached and touched; however, they were incapacitated. They exhibited lethargy, hypo-activity, and ataxia similar to that reported by Hudson et al. (1984). Their recovery was slow; at ~7 days post-baiting they were doing some moving, and they appeared normal at ~14 days.

Similar gain bait results for bobwhite quail (*Colinus virginianus*) have been reported in laboratory trials by Hines and Dimmick (1970). They observed that the repellent attributes of the black dye on oat kernels was readily apparent to quail under free-choice bait tests. Overall, they concluded that Zn_3P_2-treated oat groats posed relatively low hazard to bobwhite quail if distributed at recommended rates during a period of reasonable food abundance for quail.

FIELD STUDY – PHASE 3

In 1996, Phase 3 (the actual field study) was conducted in the Sacramento Valley at 2 sites in Sutter County, CA ~45 km apart using free-ranging pheasants. Because Ramey et al. (2000) have previously reported these results, they will be only summarized here. Both areas have some of the highest wild pheasant population densities in the state (>0.5 pheasant/acre, Hart 1990) and abundant alfalfa (Putnam 1994). One site (~2,000 ha), located southeast of the town of Meridian and adjacent to the Sacramento River, had some alfalfa fields baited with 2.0% Zn_3P_2 for vole control. The area was used predominantly for the cultivation of crops. They were, in decreasing order of acreage: rice, corn, milo, and alfalfa, intermixed with a few orchards of walnuts and persimmons, and small amounts of beans or melons. The second ~2000-ha site, located southwest of Nicolaus on the Feather River in mixed crops, was baited with placebo grain bait (0.0% Zn_3P_2) for comparison. Here, the predominant crops were rice, sugar beets, alfalfa, corn, and safflower, intermixed with some walnut orchards. Thc topography of both sites was essentially level with numerous deep (1 to 3-m) irrigation and drainage ditches in which cattails, weeds, and wild grasses sometimes grew. Pheasants were located in crops, weeds, orchards, and ditches prior to the study.

Primary crop habitats utilized by pheasants during the study included: milo (sorghum; *Sorghum vulgare*), rice (*Oryza sativa*), corn (*Zea mays*), alfalfa, and weeds. No wild pheasants were observed in alfalfa stubble fields <10 cm in height (>1,000 ac) during 270 min of trapping. In mature alfalfa (>35 cm tall), 16 wild pheasants were observed in 370 min of trapping; however, they were not easily captured because their first behavior was to run from the noise and lights of the all-terrain-vehicles (ATVs) used for capture. Only in alfalfa harvested into windrows were wild pheasants easily observed and captured. However, only 4 resident wild pheasants were captured in this habitat, although it was trapped extensively (885 min). These 4 pheasants were supplemented with 39 additional wild pheasants caught in the surrounding area, primarily in weeds, and relocated to the study sites. Additionally, 29 pen-reared pheasants were included in the study to evaluate their behavior and fate, because of the large number of pen-reared pheasants released annually for hunting. Trapping did not occur in the other crops even though many pheasants were observed or heard there, because of the potential negative economic impact to cooperators from the use of ATVs.

The study began in early September, before the next-to-last cutting of alfalfa, and continued through the first week of November 1996 (just prior to the start of hunting season). Rice was harvested at both sites during the first 3 weeks in October, and corn was harvested during the last 3 weeks. Alfalfa fields were cut for the final time for the season, dried, and baled from October 20-30. Beans were cut during the last week in September. Melons were harvested throughout October. Harvesting of various crop fields caused pheasant movements due to the removal of forage and cover as well as the harvesting activities. In addition, some rice fields were burned after harvest wherever air pollution restrictions allowed it.

Wild pheasants were captured at night by spotlighting, a successful and efficient method for obtaining pheasants under California conditions (Hart 1990; Ramey et al. 1998, 2000). An Argo, an 8-wheel amphibious ATV, or two 4-wheel ATVs, were utilized in the fields with one or more 1-million-candlepower spotlights used to locate pheasants. After spotlighting the pheasants, they were captured using large hand-held nets. The overall capture rate for pheasants in alfalfa (i.e., all stages of cultivation) was 1 bird for 289 minutes of searching with no birds observed in alfalfa stubble (<10 cm, ~500 ac), whereas the capture rate in weeds was 1 bird for every 19 minutes. After trapping in alfalfa, we trapped crop field edges, canals, ditches, and fence rows. Many pheasants were observed in these areas before they ran or flew into nearby crops that provided a safe haven from our trapping efforts prior to their harvest. Because of the small sample ($n = 4$) from alfalfa fields, nearby large weed fields were used to capture additional wild pheasants, and they were relocated to the 2 study sites. In addition, pen-reared adult pheasants raised for release at hunting clubs were purchased. All pheasants were sexed, weighed, and banded. Pen-reared pheasants were released about 3 weeks later than wild-caught birds due to their anticipated higher mortality. All 72 pheasants (native, relocated, and pen-reared) were instrumented for radio-tracking. Radio-transmitters were attached as a neck pendant design with normal operating pulse rates of either 60 or 90 ppm, and with a mortality mode of 150 ppm that was activated after 1 h of inactivity. Nineteen pheasants were lost to predation during their 6-day acclimation period, and 1 radio malfunctioned. A total of 31 wild pheasants and 21 pen-reared pheasants were studied.

Four native pheasants were released at their alfalfa capture site, and 27 relocated and 21 pen-reared pheasants were transported to either of the 2 study sites and released within hours of their capture. No pheasants died or were injured during their capture and relocation. All pheasants not caught in alfalfa were provided a choice of 3 habitats at field edges that always included alfalfa within a few meters. Following their 6-day acclimation period, 52 pheasants were monitored for habitat use by radio-tracking, using 131 tracking stations established using differentially corrected Global Positioning System (GPS) locations for triangulation purposes (67 at Meridian and 64 at Nicolaus). Birds were located at least once each day. Generally, 3 bearings were used to place each bird within an error polygon, with the vehicle located at ~90° from the transmitter for one bearing.

Some of the alfalfa fields at the Meridian site were treated with Zn_3P_2 baits for vole control; however, cooperators did not pre-bait because they believed it was too costly. During Phase 3, no sub-lethal behavioral effects were observed or detected from pheasants that could be associated with the consumption of 2.0% Zn_3P_2 bait, as occurred in Phase 2. Data indicated that habitat utilization by pen-reared pheasants was similar to wild-caught pheasants. Pheasant habitat use at the Nicolaus site (1,983 ha) and at the Meridian site (2,036 ha) was similar. Pheasants generally used the juxtaposition of mixed agricultural habitats for shelter, food, and water as similarly demonstrated by Whiteside and Guthery (1983) and seldom used alfalfa stubble following its harvest. Alfalfa use coincided with its shelter/cover potential as also reported by Hanson and Progulske (1973).

Although no pheasants were killed as a result of the Zn_3P_2 baiting, other types of mortality did occur on both study sites. During the ~40-day study at Meridian, including the Zn_3P_2 treatment in alfalfa stubble for vole control, 9 of 26 pheasants were found dead (0 native birds; 2 translocated wild birds, 14%; and 7 pen-reared, 58%). Of these, 7 died from avian or mammalian predation, and one each from harvesting operations and poaching by a hunter. During the ~40-day study at Nicolaus, 8 pheasants were found dead, victims of avian and mammalian predation. Three were translocated pheasants (20%) and 5 were pen-reared pheasants (56%).

Hessler et al. (1970) found that 4 weeks after release of pen-reared birds, mortality of radio-tagged pheasants was 81%. Mortality was greatest during the first 15 days following release than during the later 16 through 28 days. Krauss et al. (1987) compared survival of game-farm to wild birds and found that mortality averaged 76% in 1982 and 68% in 1983 for game-farm birds at 4 weeks after release, while mortality of wild birds averaged 28% and 12% at 4 weeks in 1982 and 1983, respectively. They noted that game-farm birds showed a low avoidance behavior to approach by the observer and were more susceptible to predation. In Phase 3, some pen-reared birds were naive about seeking suitable cover for protection from predators. As a result, 40% were lost during the acclimation period with the number decreasing as the birds learned avoidance behaviors.

Alfalfa use by both wild-captured and pen-reared pheasants following their release at Meridian was proportional to its availability, but not preferred as cited by Hanson and Progulske (1973). Pheasant use was confined almost entirely to the period when the alfalfa was tall and could provide cover before the alfalfa was cut (23 observations); only 2 observations were made in alfalfa stubble following Zn_3P_2 treatment. Before cutting, the alfalfa was 30 - 45 cm in height and provided good cover for pheasants. After cutting, it was only 3 - 7 cm in height and pheasants avoided these fields. Similarly at Nicolaus, pheasants utilized alfalfa fields before they were cut, baled, and became stubble. As a result, alfalfa fields were included in 7 of 24 (29%) of the pheasant home ranges at the Meridian study site and 12 of 25 (48%) at Nicolaus.

At the time of Zn_3P_2 treatment at the Meridian site and for 6 days following treatment, 6 pheasants were within 300 m (~ their mean daily movement) of the treated alfalfa fields. Two of these pheasants visited a treated field on the evening of the treatment and another pheasant the following morning. None of these pheasants demonstrated sub-lethal toxicity from the possible consumption of zinc phosphide baits broadcast at a rate of ~25.6 baits/m^2. At Nicolaus, 7 pheasants were observed at 34 locations within 300 m of the placebo-treated fields, and 5 pheasants were located within alfalfa stubble fields during the 13 days following baiting. This represented only 4% of the 133 times that pheasants were located in alfalfa fields during the entire 53 days of radio-tracking. The majority of these locations occurred when the alfalfa had grown tall enough to provide good cover.

SUMMARY

The objective of this study was to determine the extent of quail and pheasant exposure to zinc phosphide in alfalfa stubble fields during vole control programs. Results indicated quail would not eat the bait. Pheasants infrequently used alfalfa stubble fields in the fall and particularly following the last cutting of the season when the alfalfa was becoming dormant with the onset of winter. During this time, the majority of pheasants were found in milo, sugar beets, or corn before their harvest, and following their harvest the pheasants moved into weeds. After cutting, alfalfa was <10 cm in height and was not a preferred habitat of pheasants for either foraging or resting cover/shelter. Furthermore, even in baited fields (treated or placebo) the density of broadcast oat groat baits on the ground were insufficient to lure pheasants into the alfalfa fields for foraging. In conclusion, because: 1) pheasants infrequently used alfalfa stubble fields following baiting, 2) no pheasants were killed by the poison baits, and 3) no sub-lethal signs of toxicity were indicated in pheasants, the authors concluded

there is little risk to pheasants from the fall application of zinc phosphide baits to control voles and no need to have buffer areas around the treatment areas.

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