ABSENCE OF DEMONSTRABLE TOXICITY TO TURKEY VULTURES, *Cathartes aura* OF COPPER AND TUNGSTEN-TIN-BISMUTH-COMPOSITE PELLETS

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ABSTRACT

The patterns of exposure to lead and the accumulation of lead by captive-reared California condors, *Gymnogyps californianus*, released into the wild in southern California has supported the hypothesis that lead poisoning, through the ingestion of fragments of lead bullets and possibly also of lead shotgun pellets, was the principal cause of the population decline of California condors during the second half of the twentieth century. In contrast, none of the condors released to the wild in central California has been exposed to lethal amounts of lead. A self-sustaining population will not be achieved in southern California unless the use of lead-based ammunition is reduced. Copper bullets are now on the market; bullets consisting of a composite of tungsten, tin, and bismuth that matches or exceeds the ballistic qualities of lead are expected to be available in the near future. Using turkey vultures, *Cathartes aura*, as a surrogate species, we looked for toxic effects in birds that ingested copper pellets or pellets of the tungsten-tin-bismuth composite. Dosage was maintained over six weeks at a level equivalent to the ingestion of two copper bullets by a California condor. No toxic effects were detected. There were no changes in any of the hematology and blood chemistry parameters measured, nor were there any increases in the blood concentrations of copper, tungsten or bismuth; tin concentrations in the blood and liver concentrations of copper and tin increased marginally in the respective treatment groups. The geometric mean concentration of 55 ppm copper in the livers of 12 California condors that have been analyzed is 3.5 times higher than the geometric mean of 16 ppm in livers of turkey vultures used in this experiment that were not in the copper treatment group ($n = 19$). The ratio of standard deviation to the arithmetic mean of copper concentrations was also higher in the condors, 0.67 vs. 0.29, indicating a much higher variability of copper accumulation by condors than by turkey vultures. Turkey vultures may not therefore be a valid surrogate for condors in studies of copper toxicity. Moreover, whether copper poisoning contributed to or caused the death of any of four condors with high copper concentrations in the livers could not be determined; the cause of death of three of these remains unknown. No conclusion about the toxicity of copper to condors can therefore be made at the present time. The results of this experiment and all other available information indicate that bullets of the tungsten-tin-bismuth composite would not be toxic if fragments were ingested by condors or other wildlife species. Among the 38 vultures trapped for use in this experiment, five were carrying shotgun pellets in their bodies. During the 8.5 weeks of the experiment, geometric mean concentrations of lead in the blood dropped from 0.111 ppm to 0.055 ppm.

INTRODUCTION

The patterns of exposure to lead and the accumulation of lead by captive-reared California condors, *Gymnogyps californianus*, released into the wild in southern California has supported the hypothesis that lead poisoning, through the ingestion of fragments of lead bullets and possibly also of lead shotgun pellets, was the principal cause of the population decline of California condors during the second half of the twentieth century. In spite of an intensive effort to provide the birds with carcasses of domestic animals with a low lead burden, several condors in southern California have been exposed to lethal levels of lead, most likely ingested as fragments of lead bullets in carcasses of hunter-killed deer. Condor 132 succumbed from lead poisoning in January 2001. Four others, Condors 105, 98, 111 and 181 were treated by chelation after blood level concentrations above 1 ppm were recorded; most likely these birds would have died of lead poisoning without treatment (Pattee *et al.*, 1990; Redig 1984). Condors 98 and 111, both of which had high levels of 1.9 ppm lead before chelation treatment, have shown no long-term deleterious effects and are still in the wild. Condors 181 and 105 died of undetermined causes one month and 2 years, respectively, after treatment; lead exposure may therefore have contributed to the death of 181. Lead poisoning may also have contributed to the deaths of 8 other condors that disappeared with no determined cause of death. Without the monitoring of radio-tagged birds, retrieval of sick individuals and treatment by chelation therapy, the minimum number of estimated mortalities from lead poisoning in California would therefore be five, all of them in southern California (Condor Recovery Team, 2001).

In contrast, there has been no lead poisoning of the 22 condors released through 2000 into the Ventana area of Big Sur in central California, where the intensity of hunting activity appears to be lower. When expressed as estimated deaths per year if there were no retrieval and treatment of sick birds, derived from the ratio of the number of estimated deaths to the total of the years spent by all condors released to the wild in California, the current annual mortality rate from lead poisoning would be 3.8%; if the birds released in central California are excluded from the calculations, the estimated rate of mortalities from lead poisoning would be 4.9% (CRT, 2001).

At the middle of the twentieth century, the population size of California Condors is now estimated to have been about 150 birds (data of C. Koford re-analyzed by Snyder & Snyder, 1989). By 1968 and 1978 the population had dropped to about 60 and 30 birds respectively (F. Sibley, personal communication to N. Snyder; Wilbur, 1980), declining at rates of about 5% and 6.5 % a year. In 1982 the population consisted of 23 birds and in 1985 would have been 13 in the wild had not birds been captured and taken into captivity (Snyder and Snyder, 1989). The rate of population decline therefore accelerated over this interval, reaching 17% a year before all birds were taken into captivity (N. Snyder unpublished).

Rates of population decline of this magnitude of long-lived species such as condors can not be attributed to depressed productivity (Snyder & Snyder, 1989) and must therefore be caused by abnormally high rates of adult mortality. Although shooting and the poisoning of carcasses were major causes of population decline throughout the 19th century (Snyder & Snyder, 1989) there is no documentation that would indicate that they significantly increased mortality rates over 1950-85. Another mortality factor must therefore have been operating; the deaths of three birds from lead poisoning in 1984-86 (Janssen *et al.*, 1986) provided a plausible candidate. Although food shortage was never considered a factor in the population decline, the relative numbers of clean carcasses of domestic animals in the total of available carcasses may have declined over this interval, increasing the use of hunter-killed animals by the condors and accounting for any acceleration of mortality rates. The cause of the excessive mortality in 1983-85, however, remains unknown.

Without the program of supplemental feeding with relatively clean carcasses, the annual mortality rate from lead poisoning of condors in southern California would be expected to be substantially higher than the estimate of 4.9% derived above, and of the same order of magnitude as the rate of mortalities that caused the population decline over the period 1950-1985. A self-sustaining population in this area can not therefore be achieved without a reduction in the use of lead-based ammunition. As noted above, this conclusion does not necessarily apply to central California, where there has been no lead poisoning among the released condors, which have, however, continued to be supplied with 'clean' carcasses of dairy calves. Nor would it necessarily apply in areas proposed for future reintroductions such as Baja California and New Mexico. In Arizona one bird died of lead poisoning in March 2000. A massive poisoning event in June 2000 killed two condors and was the suspected cause of the deaths of another two; as many as 7 other condors were exposed to lethal levels and most likely would have died without chelation therapy (CRT, 2001). The birds had ingested shotgun pellets of three different sizes within a short time interval. The event now appears to be both unique and anomalous. An analysis of the lead exposure data from Arizona indicates that the one recorded death outside of this unique event, in a population which has generally shown only background levels of lead exposure, would not represent a level of lead exposure that would prevent the formation and survival of a self-sustaining population in Arizona (C. Woods, pers. comm.).

Nevertheless, the continuing use of lead-based ammunition anywhere in the range of California condors clearly poses a threat to the condors, either at the population level in the areas of southern California that were the principal habitat of condors during the second half of the twentieth century, or to individual birds throughout their range. A requirement that non-toxic ammunition be used in government programs and a phasing-in of the use of non-toxic ammunition by the hunting community, at least in selected critical areas, would clearly benefit condors. To date, however, the choice and availability of non-toxic bullets have been limited.

The choices and availability of non-toxic shot, however, have expanded since the decision was made to phase out the use of lead-shot ammunition over marshes and other wetlands in response to an unacceptable level of damage to wildlife. The ingestion by waterfowl of lead-shot pellets caused annual mortalities of 1-3 million birds from lead poisoning (Bellrose,1959; Sanderson & Bellrose, 1986; United States Fish and Wildlife Service, 1986). Swans, which frequently also ingest lead fishing sinkers, have been particularly affected by lead poisoning; many mortalities of the Trumpeter Swan, *Cygnus buccinator*, have been documented in the northwestern United States and in British Columbia (Kendall & Driver, 1982; Blus *et al.*, 1989). Globally, a minimum of 10,000 swans of six species in 14 countries have died from the ingestion of lead shot pellets or of sinkers (Blus, 1994). Scavengers that consume carcasses of waterfowl and predators of birds wounded or incapacitated by lead shot have in turn been poisoned; in North America the Bald Eagle, *Haliaeetus leucocephalus*, suffered a high rate of mortalities from lead poisoning following the ingestion of lead pellets (Mulhern *et al.*, 1970; Kaiser *et al.*, 1980; Reichel *et al.*, 1984; Pattee *et al.*, 1981; Pattee & Hennes, 1983; Redig, 1984). The ending of the use of lead shot in wetland areas has substantially reduced the damage to these species of wildlife.

The steel shot introduced in 1991, however, proved to be initially unpopular in the hunting community. The low density of steel, 7.86 gm/cc^{-1} is only 71% of the average density of lead shot of 11.1 gm/cc^{-1}, reducing ballistic performance; its hardness caused severe damage to the barrels of shotguns then in use. Modifications in shotgun design have since improved the performance of steel shot and the use of larger pellets can compensate for the lower density of steel (Lowry, 1999).

The use of bismuth shot, containing 3% tin to reduce frangibility, was approved by the FWS in January 1997, following earlier temporary approval for use in the 1994-95 and 1995-96 hunting seasons.. Its density is 9.69, and its hardness only slightly higher than that of lead. Tungsten-iron shot consisting of a 40% iron, 60% tungsten alloy has a density of 10.4, only slightly lower than that of lead; it was given preliminary approval for use in the 1997-98 season. Tungsten polymer shot of the Federal Cartridge Co., in which particles of tungsten are suspended in a nylon polymer binder, has a density equivalent to that of lead; the nylon reduces the abrasive effects of tungsten on the shotgun barrel. It was initially approved for the 1998-99 season. A tungsten-matrix shot, in which tungsten particles are also suspended in a polymer matrix, has been introduced by the Kent Cartridge Co. (Lowry, 1999; FR 63(143)40074-40077). Research and technology have therefore produced products that match the desirable properties of lead without producing any demonstrable deleterious environmental effects.

Regulations requiring the use of non-toxic shot in marshes and other wetlands have so far not been applied to upland areas. Two recent reviews on hazards of shot-derived lead to upland wildlife (Scheuhammer & Norris, 1996; Kendall *et al.*, 1996) summarize the evidence that many species in addition to condors are subject to lead poisoning following the ingestion of lead shot used in upland areas.

The "Green Bullet" program of the US Department of Defense aims to replace the lead-based ammunition used at its firing ranges with a non-toxic alternative, largely because of the cost of cleaning up lead-contaminated soil, but also for health reasons. Dust and vaporized lead present a severe health risk to personnel training in indoor ranges without adequate ventilation; lead is inhaled and assimilated into the body. 150 Army Reserve and over 600 National Guard indoor ranges are currently closed because of the dangers posed by the use of lead ammunition (htpp://www.pica.army.mil/greenammo). The development by the military of non-toxic lead-free bullets that will be produced in large quantities and ultimately made available to the hunting communities is expected in the future to reduce the exposure to lead of condors and other scavenging wildlife species.

Copper bullets, containing approximately 5 % zinc, have been available on the commercial market for several years and have been successfully and satisfactorily used in a program to remove feral goats and pigs from southern California islands where the use of lead ammunition would threaten the introduced population of bald eagles (D. Garcelon, pers. comm.). Since a copper-zinc alloy of this approximate composition has been traditionally used in copper-jacketed bullets, a shift towards the use of lead-free copper bullets would not therefore appear to pose a new threat to condors. Yet the death of condors in both Arizona and California from causes not determined with apparently high concentrations of copper in their livers (CRT, 2001) has raised questions about the impact of copper on condors. Unlike lead, bismuth, tin, and tungsten, copper is an essential element; it is a component of the critically important enzyme cytochrome C oxidase (Yoshikawa *et al.*, 1998). Like mercury and lead, however, it readily binds with sulfhydryl groups of proteins, thereby inactivating them; intracellular transport is accomplished by "chaperone" molecules that deliver copper ions to the sites where they are incorporated into the enzymes (Pufahl *et al.*, 1997). Excess copper, and copper in the wrong place, are therefore expected to result in toxicity. Numerous cases of copper toxicosis of domestic animals have been described in the literature, which also records at least two cases of copper poisoning of waterfowl, Canada geese, *Branta canadensis* (Henderson & Winterfield, 1975), and mute swans, *Cygnus olor* (Kobayashi *et al.*, 1992). Copper may also have caused mortalities of mute swans in Denmark; concentrations in excess of 1,000 ppm were recorded in livers (Clausen & Wolstrup, 1978).

A more promising substitute for lead in the manufacture of bullets is produced by suspending finely-powdered tungsten in an molten bismuth-tin alloy. Densities and hardness equivalent to lead can be achieved with a composition of 55% tungsten, 43% tin and 2% bismuth, here referred to as TTC, tungsten-tin composite (Victor C. Oltrogge, Denver, CO; U.S. patent 5,279,787; other patents pending). Toxicity tests with game-farm mallards, *Anas platyrhynchos*, orally dosed with TTC pellets showed no changes in 23 hematology and blood chemistry parameters between dosed and undosed birds, and no detectable uptake of tungsten, tin, or bismuth (Ringelman *et al.*, 1993). No data, however, on the potential toxicity of TTC composite to condors and related species have been published.

In this paper we report the results of an experiment in which pellets of copper and of a tungsten-tin-bismuth composite (55:43:2) were fed to turkey vultures, *Cathartes aura*, selected as a surrogate species for the California condor, both belonging to the Family Cathartidae of New-World vultures. The TTC pellets were kindly provided by Mr. Oltrogge for our use in this experiment.

MATERIALS AND METHODS

An initial group of 6 turkey vultures was trapped on Rancho Mission Viejo in Orange County in southern California for a feasibility study, primarily to determine whether pellets would be retained or regurgitated, and whether they could be detected by whole-body x-rays with a MinXray 803 field x-ray unit. A 7' x 7' walk-in trap, baited with a carcass and a decoy turkey vulture on loan from a local rehabilitation center, was used to capture wild birds. Once the first wild bird entered the trap, others readily followed. Vultures were housed individually in 34" x 34" cages on Rancho Mission Viejo. Removable floors permitted cleaning and collection of feces.

Pellets of pure copper were purchased from Fisher Scientific; mean weight of a subsample (n = 423) used in the study was 0.095 gm. Mean weight of a subsample of the TTC pellets (n = 195) was 0.205 gm.

Dosage was determined from the assumption that at most a condor would ingest and temporarily retain a maximum of the equivalent of two copper bullets, each weighing 180 grains or 11.7 gm. Dosage in a 10 kg condor would therefore be 2.3 gm/kg. This dosage was used for both kinds of pellets ingested by the vultures, with adjustments for the weights of individual birds.

In the feasibility study vultures were maintained on a bovine heart diet and copper pellets were used. At least two thirds of the pellets stuffed inside chicken hearts were ingested and could be detected by the x-ray unit. Pellets tended to clump together in the digestive tract; counts therefore are minimal. At the end of Week 1, 17 clumps were in the crops, 87 in the intestine, total 104;at the end of Week 2, 13 clumps were in the crops, 66 in the intestine, 23 in the feces, total 102; after Week 3, none were in the crops, 66 were in the intestine, and 2 were in the feces, total 81. At week 4.5, 81 pellets were recovered with the feces. The feasibility study therefore demonstrated that the birds would ingest and temporarily retain pellets, but would have to be redosed periodically to maintain dosage. It also demonstrated that pellets would eventually be voided. The birds were transferred back to the walk-in trap at Week 4.5 to permit exercise before release at week 7. At that time, one bird had one pellet in the intestine. Birds flew away with no symptoms of harm. During the weekly handling, at least 5 of the 6 birds regurgitated a greenish fluid on at least one occasion. Their feces were also green.

A total of 32 birds was captured for the experiment in early December 1996, 11 on Rancho Mission Viejo, the remainder in San Luis Obispo County, California. After at least a week of acclimation, the birds were weighed and x-rayed on 16 December 1996, which was thereafter designated as Week 0 and Day 1 of Week 1. 5-7 ml of blood were taken from a brachial vein and divided into three aliquots: 1) EDTA treated tube for blood parameters; 2) sterile with no additive for metal chemistry; 3) a clotting tube for preparation of serum. Vulture D1 contained 9 shotgun pellets in the chest and abdominal areas and was released after a wedge liver biopsy sample was taken (It was observed at Morro Bay west of San Luis Obispo 4 years later in March 2001). Vulture D2 was selected for an additional practice run for obtaining wedge liver biopsies, and was also released. The remaining thirty of the birds were randomly assorted into three groups. The birds were not fed for two days. Groups B and C (n = 10 each) were dosed with TTC and Cu pellets, respectively, inside chicken hearts that had been closed with surgical sutures; Group A served as control (n = 10). Birds were maintained at the Starr Ranch Sanctuary of the National Audubon Society in Rancho Santa Margarita, CA. The cages were larger than those used in the feasibility study, with dimensions of 36" x 36" x 36".

Blood samples and x-rays were taken weekly except that the last sample was taken at 8.5 weeks (Feb. 13, 1997). Retention of pellets was determined from counts of the numbers of pellets visible in the x-ray photographs. Treatment birds were redosed individually each week to restore the 2.3 g/kg dosage.

At Week 0 the vultures weighed 1.685 kg ± 0.130 (SD). The previous diet of laboratory rats was changed to a protein mixture for zoo animals to minimize regurgitation. Food consumption dropped sharply; average weight loss at Week I was 100 gm. Death of one of the control birds prompted resumption of the laboratory rat diet. This bird was later shown to have abnormally high copper levels in the blood, higher than any recorded throughout the experiment, and had low blood levels of iron and zinc (below). Regurgitation of rat remains removed all pellets from the crops of all treatment birds. The birds accepted a diet of bovine hearts and lost weight was regained by the end of Week II; redosing was accomplished by insertion of pellets inside small cubes of heart tissue that the birds swallowed whole. The projected 6-week study was extended an additional two weeks. Liver biopsy wedge samples were taken at the end of the experiment: 12 birds, four from each group were sampled on February 13 and 15, five from each group on February 14 (Week 8.5); the remaining three birds were sampled on February 21 1997. In the data summaries and analysis, the data from the last sampling are combined with those of

Week 8.5.

Blood plasma and hematology parameters were measured at Antech Diagnostics, Irvine, CA, and consisted of glucose, total protein, uric acid, lactic acid dehydrogenase, aspartate amino transferase, creatine phosphokinase, red blood cells, hematocrit, and white blood cells (heterophils, lymphocytes, monocytes, basophils, and eosinophils).

Copper, tungsten, tin, bismuth, iron, zinc and lead concentrations in blood of the Weeks 0, 5 and 8.5 samplings and in the liver biopsies were determined by West Coast Analytical Service, Inc., Santa Fe Springs, CA by ICPMS (Inductively Coupled Plasma-Mass Spectrometry). The laboratory maintains separate Quality Assurance and Quality Control programs that conform with EPA and FDA Good Laboratory Practices. Two blind samples were submitted, nominal concentrations are in parenthesis: 1) National Institute of Standards and Technology Standard Reference Material 1974, homogenized mussel tissue, micrograms/gm dry weight: copper, 1.41 (1.14); zinc, 9.55 (11.3); iron, 70.5 (61.8); lead, 1.44 (1.20); 2) Elemental Analysis Standard for copper as copper nitrate (Aldrich Chemical Co., Inc.), micrograms/ml: < 0.006 (0.00); 0.99 (1.00); 2.48 (2.53); 1.72 (1.76). Accuracy of the analyses was considered satisfactory.

The liver wedge biopsies were taken by veterinarians at the San Diego Wild Animal Park, Escondido, CA and the Serrano Animal and Bird Hospital, Lake Forest, CA. Aliquots were taken for: 1) metal quantification by ICPMS; 2) routine histopathology, undertaken by Dr. Linda Lowenstine D.V.M. at the University of California, Davis; tissues were fixed in 10% buffered formalin, and embedded in paraffin; sections were stained with hematoxylin and eosin and with Perl's stain for iron; 3) transmission electron and light microscopy at California State University, Long Beach. Tissues were fixed at room temperature for 2 hours in 1% formaldehyde and 2% glutaraldehyde prepared in 0.05 M Millonigs phosphate buffer (pH 7.4). Samples were washed in buffer and fixed for 1 hour in a secondary fixative of 2% osmium tetroxide in Millonigs-buffer. The tissues were then dehydrated with graded ethanol series (15 minutes each) and propylene oxide (2x10 minutes each). The dehydrated samples were embedded in Spurr epoxy resin and cured in a 70°C vacuum oven for 18 hours. Sections approximately 500 nm and 40-80 nm in thickness were cut using a Sorvall MT-2 Ultra Microtome for light microscopy (LM) and electron microscopy respectively. LM sections were stained with Toluidine Blue (0.5%) in 1.0% Borax. The ultrathin sections for TEM were stained with saturated uranyl acetate (5 minutes) followed with lead citrate (3 minutes) and viewed in a JEOL 1200 EXII TEM. TEM micrographs were taken at 40 and 80kV.

Scanning electron micrographs were taken at 25kV, using a JEOL T200 SEM, of unused pellets and acetone-washed pellets collected from the feces to determine the degree of surface corrosion.

Statistical analyses were undertaken with the Statgraphics Program.

At the conclusion of the experiment the birds were transferred back to the trapping cage for exercise prior to their successful release to the wild.

RESULTS

During Week II (Days 8 - 14), after the diet was changed from the zoo protein mixture to beef hearts, weights of the birds returned to their previous levels and showed no significant changes thereafter. No significant changes in blood chemistry or hematology parameters were recorded among groups or over time throughout the experiment.

All of the birds regurgitated all of the pellets in their crops along with fur and bones of the rats provided them to stop their weight loss on the zoo protein diet. After weights were stabilized with a beef heart diet and the birds were redosed, retention of the pellets was highly variable among the birds and over time with pellet clumps remaining longer times in the crop or intestine in some birds than in others.

Blood copper levels in the treatment group did not change over the experiment ($P = 0.56$, simple regression) and at Week 8.5 were not significantly different from the control or TTC groups ($P = 0.14$; Table 1). At Week 8.5, geometric mean concentrations in the treatment group, with the interval of one standard deviation, were 0.25 ppm (0.20 - 0.31; n = 10) vs 0.28 ppm (0.21 - 0.38; n = 19) in the combined control and TTC groups (Table 1). The control bird that died had a blood copper concentration at Week 0 of 0.86 ppm, beyond the range recorded in the other birds, and substantially higher than 0.41, the upper limit of the 95% confidence interval of the geometric mean of all other copper concentrations at Week 0; iron and zinc concentrations were in the lowest 10 % of recorded values, 243 and 4.2 ppm, respectively. The copper concentration in this bird must therefore be considered abnormally high for turkey vultures.

Although copper concentrations in the blood of the treatment group at Week 8.5 were not elevated above those in the combined control and TTC treatment groups, those in the liver were higher (*t* tests, $P = 0.040$, arithmetic distribution; $P = 0.019$, logarithmic distribution): 20.5 ppm (16.6 - 25.2; n = 10) vs 16.2 ppm (12.0 - 21.9; n = 19), all geometric means with interval of one standard deviation. Exposure to copper therefore resulted in a low level of copper accumulation in the liver, although concentrations did not increase in the blood.

A green regurgitate and green feces were consistently observed in the copper treatment group, and were not observed in birds of the other groups. Further evidence of the release of copper from the pellets was provided by electron microscopy, which showed erosion of the surfaces of pellets that had been voided with the feces.

Zinc concentrations in blood did not change over time nor were there any differences among groups in either blood or livers (Table 1).

Iron concentrations also did not change significantly over time nor were there differences in blood levels among the groups. In the copper treatment group, however, there was an apparent, but non-significant tendency towards higher concentrations in blood (357 ppm, 302-422, n = 10, vs. 272 ppm, 204-362, n = 19; Kolmogorov-Smirnov statistic 1.28, $P = 0.08$); iron concentrations, however, were lower in the livers of the copper treatment group at Week 8,5 (*t* tests, 142 ppm, 94-215, n = 10, vs. 203 ppm, 142-280, n = 19; $P = 0.040$ for the arithmetic distributions and 0.019 for the log-normal distributions, geometric means with the interval of one standard deviation).

Histological examination indicated higher than normal concentrations of iron in the livers of the 2 'D' birds from which wedge liver samples had been taken in the feasibility study but which were not included in the experiment; D1, with 9 shotgun pellets in muscle tissue, had 684 ppm iron in the liver, D2 had 477 ppm of iron. Both concentrations were above the range found in the other birds; geometric mean concentration of iron in the liver at the end of the experiment was 180 ppm (121 - 266, interval of one standard deviation). Lead concentration in the liver of D1 was high (below).

Tin concentrations increased marginally in the blood of birds dosed with TTC. For initial statistical analysis, values below the detection limit were recorded as one half of that value (Table 1). In the control group all measurements were below detection limits except 3 at the detection limit of 0.003 ppm at Week 8.5. In the copper treatment group all tin concentrations were below detection limits at each sampling event. In the TTC treatment group, one bird was at the detection limit of 0.003 ppm at Week 0, the others were below. At Week 5, 2 birds had tin concentrations at twice the detection limit; the others varied between 0.003 and 0.006 ppm. At Week 8.5, 7 birds were at the detection limit, the others 2, 3, and 4 times higher, respectively.

In the livers, tin concentrations in the control group were below detection limits ranging between 0.02 and 0.07 ppm; 4 were above at a geometric mean concentration of 0.65 ppm. In the copper treatment group, 8 were below the detection limit and two above with a mean concentration of 0.04 ppm. In the TTC treatment group, all values were 11 ± 8 times above the detection limits with a mean value of 0.25 ppm. Tin therefore accumulated in the livers of birds on the TTC diet, but the concentration reached is not considered toxicologically significant (Schafer & Femfert, 1984).

Concentrations of both tungsten and bismuth were below detection limits in all samples;

there was therefore no evidence of accumulation of these elements. Electron microscopy, however, showed that the surfaces of the TTC pellets that had passed through a bird were heavily corroded, showing that the birds had been exposed to the three metals in the composite.

Lead concentration in the livers of the copper treatment group at Week 8,5 were lower than those in the other groups, but the differences were not significant (Komogorov-Smirnov test, $P =$ 0.145). Blood lead concentrations at Week 0 were highly variable, ranging from 0.03 ppm to 0.91 ppm with a geometric mean of 0.111; 8 were above 0.2 ppm, the level considered to be the threshold for an anthropogenic component in condors (CRT, 2001) . Over the 8 weeks of captivity, mean levels fell by half (Table 1). One of the birds in the feasibility study was carrying two shotgun pellets; as noted above, D1 had 9 shotgun pellets in the chest and abdominal areas. The liver lead concentration, 0.38 ppm. was exceeded by only two values at Week 8.5 in the livers of the birds used in the experiment, 0.71 ppm and 1.18 ppm. The latter value was associated with the highest recorded blood concentration, 0.91 ppm, at Week 0. Blood lead concentrations of D1 were not measured. One of the birds of the control group was carrying one pellet; in the TTC group, one had one pellet, another had three.

DISCUSSION

Of the total of 38 birds trapped for the feasibility and experimental studies, five were carrying lead pellets in their tissues, indicating a continuing high level of random shooting in southern California. The sighting in the wild 4 years later of the turkey vulture carrying 9 pellets in its body in December, 1996 indicates that such a burden is not necessarily toxic. Since deer are among the wildlife species likely to be subject to random indiscriminate shooting, some are likely to be carrying shotgun pellets, posing a hazard to condors if deer are later killed but not retrieved by hunters.

The removal of all pellets from the crops of the treatment birds with regurgitation of the fur and bones of laboratory rats indicates that scavengers which regurgitate undigestible remains are less likely to be poisoned by fragments of lead or shot pellets than are condors which consume only 'soft' tissues.

The absence of any observable toxic or other effects of the TTC pellets on turkey vultures is consistent with the results of previous studies of the toxicity of bismuth and tungsten (Nell *et al.*, 1981; Sanderson *et al.*, 1992; Ringelman *et al.*, 1993; Kraabel *et al.*, 1996; Kelly *et al.*, 1998) and with the conclusion of a review of the toxicity of metallic tin (Schafer & Femfert, 1984). Moreover, the extensive review process for approval of use of the several non-toxic shotpellets on the market failed to find any evidence of subaccute longer-term toxicity (Lowry, 1999). It is very unlikely, therefore, that the use of TTC ammunition would result in any harm to condors and other scavenging species.

On June 30 1999, Condor G78, SB 178, a juvenile released at Lion Canyon on March 24 of that year, was recovered dead about 5 miles northeast of Lion Canyon, having lost 30 % of its body weight in two weeks. Copper concentrations were 75 ppm in the liver, 77 ppm in the kidney. Iron concentrations were also high, 843 ppm in the liver, 867 ppm in the kidney (Necroscopy Report, San Diego Zoological Society). In contrast, the mean copper concentration in the livers of the combined control and TTC-treatment groups if turkey vultures was 16 ppm, mean iron concentration was 180 ppm (above, Table 1). Wiemeyer *et al.* (1983) reported a concentration of 87 ppm in the liver of a condor recovered in Kern county in 1976. This concentration is about twenty times higher than those recorded in adult ospreys, *Pandion haliaetus* (Wiemeyer *et al.*, 1980) and in three species of Australian raptors (Beck, 1956). In 2000, concentrations of 95, 120 and 181 ppm copper were recorded in the livers of one condor from California and two from Arizona. Death of one of the Arizona birds, with 181 ppm in the liver, has been attributed to lead poisoning on the basis of a lead concentration in the liver of 17 ppm. Causes of death of the other condors with high concentrations of copper could not be determined, raising the question whether copper poisoning was a contributing factor. All available data on the concentrations of copper in the livers of condors (Table 2) indicate that condors appear to accumulate higher levels of copper than turkey vultures and at least several species of raptors. The geometric mean concentration of 55 ppm copper in the livers of the 12

California condors that have been analyzed is 3.5 times higher than the geometric mean of 16 ppm in livers of turkey vultures used in this experiment that were not in the copper treatment group (n = 19). The ratio of standard deviation to the arithmetic mean of copper concentrations was also higher in the condors, 0.67 vs. 0.29, indicating a much higher variability of copper accumulation by condors than by turkey vultures. Turkey vultures may not therefore be a valid surrogate for condors in studies of copper toxicity. Although condors have been exposed to the copper of copper-jacketed bullets without suspicion of harm, determination whether excess copper may contribute to the deaths of condors and an assessment of the impact of excess copper on condors are therefore prerequisite to any recommendations that copper bullets replace bullets containing lead within any or all portions of the condor range.

The results of this experiment and all other available information indicate that bullets of the tungsten-tin-bismuth composite would not be toxic if fragments were ingested by condors or other wildlife species. The long-term survival in the wild of a self-sustaining population of California condors in southern California is clearly dependent upon the replacement of lead bullets with TTC or another form of non-toxic ammunition.

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Table 1, page 1. Metals in the Blood and Liver: Turkey Vultures Dosed with Copper and TTC Pellets

Parts per million, geometric means with interval of one standard deviation

		Week (s)	Group A Control n = 9		Group B Tungsten-Tin-Bismut n = 10		Group C Copper n = 10		All Groups n = 29	
Copper	Blood	0	0.33	(.23-.47)	0.31	(.20-.46)	0.24	(.19-.29)	0.29	(.20-.41)
		5	0.24	(.20-.30)	0.25	(.18-.35)	0.27	(.23-.31)		
		8.5	0.28	(.21-.39)	0.30	(.21-.38)	0.25	(.20-.31)		
	Changes	0-5	-0.09	+/- 0.13	-0.06	+/- 0.17	0.03	+/- 0.04		
		0-8.5	-0.05	+/- 0.06	-0.04	+/- 0.12	0.01	+/- 0.05		
	Liver	8.5	17.6	(13.2-23.3)	15	(11.2-20.2)	20.5	(16.6-25.2)		
Zinc	Blood	0	6.4	(5.5-7.6)	5.5	(4.5-6.8)	5.2	(4.5-6.1)	5.7	(4.7-6.9)
		5	6.2	(5.2-7.4)	6.0	(5.0-7.2)	6.7	(6.1-7.4)		
		8.5	5.9	(5.3-6.6)	6.1	(5.3-7.1)	6.2	(5.4-7.1)		
	Liver	8.5	42.8	(35.0-52.4)	40.9	(37.8-44.1)	40.2	(36.1-44.9)	41.2	(35.9-47.4)
Iron	Blood	0	328	(302-356)	313	(270-363)	301	(257-354)	313	(272-361)
		5	325	(281-377)	294	(229-377)	341	(313-373)	319	(265-385)
		8.5	260	(183-368)	283	(230-349)	357	(302-422)	299	(225-397)
	Changes	0-5	0.0	+/- 50	-13	+/- 98	37	+/- 57	8	+/- 77
		0-8.5	-54	+/- 69	-27	+/- 88	64	+/- 46	-6	+/- 84
	Liver	8.5	222	(172-285)	188	(132-268)	142	(94-215)	180	(121-266)

Table 1, page 2. Metals in the Blood and Liver: Turkey Vultures Dosed with Copper and TTC Pellets

Parts per million, geometric means with interval of one standard deviation

		Week (s)	Group A Control n = 9		Group B Tungsten-Tin-Bismutl n = 10		Group C Copper n = 10		All Groups n = 29	
Tin	Blood	0	0.0015		0.0016		0.0015			
		5	0.0019		0.0034		0.0015			
		8.5	0.0015		0.0026		0.0015			
	Liver	8.5	0.041 See Text	(.019-.087)	0.25 See Text	(.13-.49)	0.015 See Text	(.008-.028)		
Tungsten	Blood	0	0.0017		0.003		0.0015			
		5	0.0015		0.004		0.0015			
		8.5	0.0010		0.001		0.001			
	Liver	8.5	0.056	(.005-.62)	0.026	(.004-.12)	0.025	(.002-.30)		
Bismuth	Blood	0	0.001		0.001		0.001			
		5	0.001		0.001		0.001			
		8.5	0.001		0.001		0.001			
	Liver	8.5	0.0027		0.002		0.0023			
Lead	Blood	0	0.108	(.044-.267)	0.130	(.067-.254)	0.096	(.036-.257)	0.111	(.046-.265)
		5	0.083	(.044-.155)	0.079	(.055-.113)	0.070	(.033-.147)	0.077	(.042-.140)
		8.5	0.051	(.027-.094)	0.054	(.037-.080)	0.06	(.028-.13)	0.055	(.030-.102)
	Changes	0-5	-0.06	+/- 0.10	-0.07	+/-.08	-0.09	+/-.19	-0.07	+/- .15
		0-8.5	-0.14	+/- 0.14	-0.1	+/-.09	-0.09	+/- 0.20	-0.10	+/- .15
	Liver	8.5	0.112	(.049-.26)	0.108	(.70-.17)	0.071	(.022-.23)	0.098	(.040-.239)

Week 8.5: 12 birds, 4 from each group, sampled on 13 February, 1997; 15 birds, five from each group sampled on 14 February; remaining three birds sampled on 21 February.