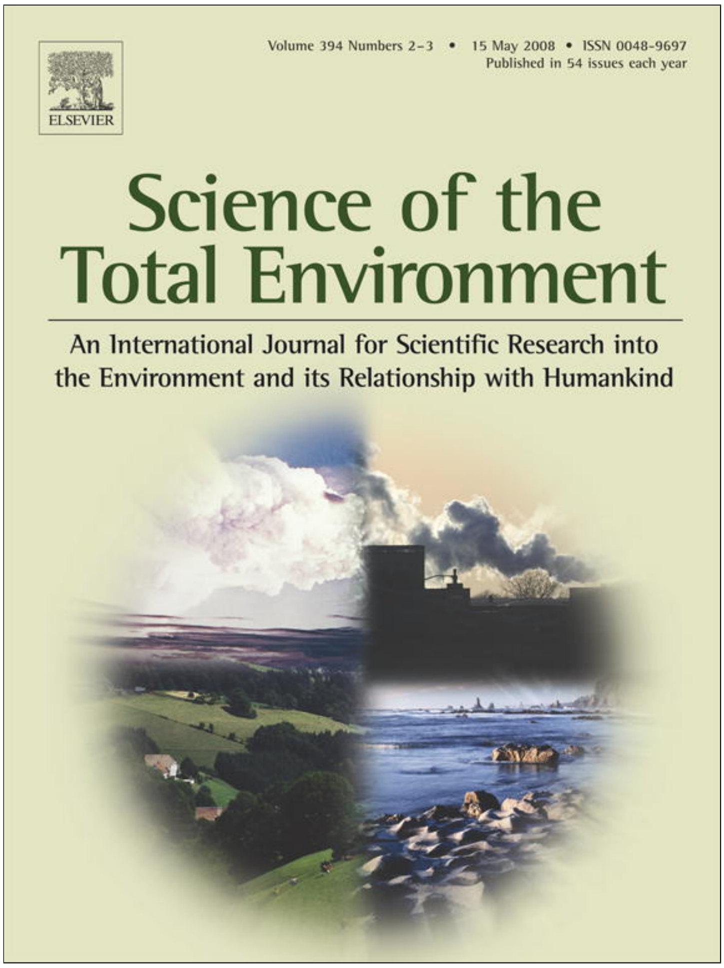

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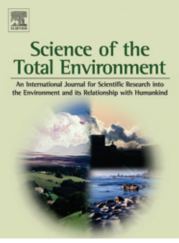

Dissolution of copper, tin, and iron from sintered tungsten–bronze spheres in a simulated avian gizzard, and an assessment of their potential toxicity to birds

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ABSTRACT

The rates of dissolution of copper, tin, and iron from sintered tungsten–bronze spheres (51.1%W, 44.4%Cu, 3.9%Sn, 0.6%Fe, by mass) were measured in an *in vitro* simulated avian gizzard at pH 2.0, and 42C. Most of the spheres had disintegrated completely to a fine powder by day 14. Dissolution of copper, tin, and iron from the spheres was linear over time; all $r>0.974$; all $P<0.001$. The mean rate of release of copper, tin, and iron was 30.4 mg, 2.74 mg, and 0.38 mg per g tungsten–bronze per day, respectively. These rates of metal release were compared to those in published studies to determine whether the simultaneous ingestion of eight spheres of 3.48 mm diameter would pose a toxic risk to birds. The potential absorption rates of iron and tin (0.54 mg Fe/day, and 3.89 mg Sn/day) from eight tungsten–bronze spheres of total mass 1.42 g would not prove toxic, based on empirical studies of tin and iron ingestion in waterfowl. The release of 43.17 mg copper/day from eight tungsten–bronze spheres, while exceeding the daily copper requirements of domesticated birds, is far below the levels of copper known to cause copper toxicosis in birds. We conclude that sintered tungsten–bronze material made into gunshot, fishing weights, or wheel balance weights, would not pose a toxic risk to wild birds when ingested.

1. Introduction

The extent of fatal lead poisoning of birds from the ingestion of discharged lead shot has led to an ever-growing list of nations regulating bans on the use of lead shot (Beintema, 2001; Thomas and Guitart, 2005). This, in turn, has induced the development of diverse lead substitutes containing iron, tungsten, bismuth, tin, and copper in different proprietary combinations (USFWS, 2006). In Canada and the USA it is mandatory that such lead substitutes pass a rigorous toxicological screening before being approved for use (USFWS, 1997; Thomas, 2003), but no other countries require such an evaluation.

The need to regulate lead in shot is being extended to other applications of lead, as in fishing weights (Perry, 1994; Scheuhammer et al., 2003), but in this case, no nation requires mandatory toxicological screening of lead substitutes (Thomas and Guitart, 2003). Sintered tungsten–bronze is a new material devised and approved, initially, for shot (USFWS, 2004), but has physical and manufacturing properties that dispose it to use in fishing weights and automotive wheel balance weights.

In both recreational shooting and angling it is inevitable that discharged shot and lost fishing weights will be ingested by waterfowl and other aquatic avian species (Perry, 1994; Twiss and Thomas, 2003). In situations where birds have been wounded by gunfire, and then fall prey to both avian and mammalian

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Table 1 – Chemical composition of tungsten–bronze spheres used in dissolution tests

Sample replicate	Mass (mg)	Copper %	Tin %	Iron %	Tungsten %
Mean ± standard deviation	177.7 ± 2.9	44.4 ± 0.55	3.9 ± 0.12	0.60 ± 0.016	51.1 ± 0.56

The values are % by mass, based on five randomly-selected batches. The level of tungsten was measured by difference.

predators and carrion feeders, shot in the tissues will enter the gut of the animal, and may prove toxic (Kramer and Redig, 1997; Fisher et al., 2006). Lead balance weights are lost from vehicle wheels, pollute roadside habitats, and increase the lead loading of human urban environments (Root, 2000). Thus, there needs to be a process to evaluate the potential toxicity of ingested candidate lead substitutes to animals, especially outside of the USA and Canada. The passage of the European Directive on Chemicals in the Environment in 2007 (CEC, 2006) means that every new chemical material that may be released to the European environment must be assessed in terms of its potential toxicity.

The release of copper from sintered tungsten–bronze spheres under different pH conditions was measured by Thomas et al. (2007a), and shown to pose no toxic risks to even the most sensitive aquatic organisms. Determination of the non-toxicity of any substitute for lead shot and sinkers also requires that the release of metal salts under the prevailing pH in waterfowl gizzards be measured and compared with known levels that pose toxic risks to birds. This *in vitro* study was designed to simulate the chemical dissolution that ingested tungsten–bronze spheres would experience inside an avian gizzard. The amount of copper, tin, and iron liberated from the spheres was measured over time and related to their potential toxicity to birds. The only metals of potential concern in this study are copper and tin, since tungsten and iron have already been shown to be non-toxic to waterbirds when ingested (Mitchell et al., 2001a,b,c). Nonetheless, a discussion of the potential dissolution and absorption of tungsten salts from the tungsten–bronze spheres is provided in light of the findings of Mitchell et al. (2001a,b,c).

2. Materials and methods

Both physical and chemical break-up of ingested materials occurs in the highly acid medium of the gizzard (ventriculus). Kimball and Munir (1971) established an *in vitro* protocol to measure the dissolution of metals in simulated waterfowl gizzards. This protocol was used to measure the actual rates of release of aqueous copper, iron, and tin from tungsten–bronze spheres into the synthetic medium. The experimental work was performed in the laboratories of the International Tin Research Institute, UK. The digestive medium was 1 M sodium chloride solution containing 20 g/l of Pepsin A, and the pH of the solution was adjusted to 2.0 using hydrochloric acid (Kimball and Munir, 1971). All reagents were of analytical grade (Merck), whether used in the dissolution medium or for subsequent ICPOES metal analyses.

The experiment comprised an experimental treatment using tungsten–bronze spheres (3.48 mm diameter), a glass bead (3 mm diameter) control, a pure copper sphere (3.4 mm) control, a tin sphere (3.3 mm diameter) control, and an iron sphere (3.3 mm diameter) control. The composition of the tungsten–bronze sphere was 51.1% tungsten, 44.4% copper, 3.9% tin, and 0.6% iron (Table 1). The trace elemental composition of the sphere, and the particle size distribution of the bronze and tungsten components are given in Thomas et al. (2007a). The presence of iron in the formula serves as a densifier of the material, and also serves to render the material magnetic, as per the legal requirements of the USFWS (2006).

There were five replicates within each control and experimental treatment. A single tungsten–bronze sphere was weighed and placed in a 100-ml Pyrex bottle fitted with a loose screw-top closure. One hundred ml of the acid medium was added, together with a Teflon magnetic stirring bar. The same procedure was used for the glass bead, tin, iron, and copper sphere controls. The bottles were placed on magnetic stirrers inside a thermostatically-controlled oven set at 42 °C, and the samples were stirred continuously for 14 days. Each bottle allowed air to equilibrate with the dissolution medium for the entire 14-day period. This time period was selected because it was consistent with the known time taken for tungsten–bronze spheres to disintegrate, completely, in the simulated gizzard (Table 2). A third experimental treatment was used to measure the impact of the presence of iron in the tungsten–bronze formula on the physical integrity of the spheres and potential dissolution rates of copper and tin by the acid medium. In this case, the tungsten–bronze pellet did not contain 0.6% iron, but had the composition 3.9% tin, 44.4% copper, and 51.7% tungsten. This experimental treatment was subjected to the same dissolution conditions as the tungsten–bronze spheres containing iron.

Table 2 – The mass of individual tungsten–bronze spheres before and after 14 days of immersion in the dissolution medium (A), and (B), the mass of individual tungsten–bronze spheres (containing 0% iron) before and after dissolution in the acid medium for 14 days

A	Tungsten–bronze spheres (0.6% iron)			
	Mass before test mg	Mass after test mg	Loss of mass mg	%
Mean value ± 1 SD	173.7 ± 4.8	1.96^a ± 2.9	171.7 ± 4.2	98.9 ± 1.6
B	**Tungsten–bronze spheres (0% iron)**			
	Mass before test mg	Mass after test mg	Loss of mass mg	%
Mean ± 1 SD	125.74 ± 1.76	98.97 ± 9.61	26.77 ± 9.15	21.31 ± 6.00

N = 5 for each mean value.

[a] Denotes that no measurable pieces were in three of the five reaction bottles.

Table 3 – The release of copper, tin, and iron from their respective pure metal controls by the dissolution medium over 14 days

Treatment	Time, days									
	1	2	3	4	7	8	9	10	11	14
Pure copper spheres	40.4±7.0	105.8±7.2	159.5±11.1	181.5±13.2	197.8±14.0	207.7±16.5	222.4±17.1	225.9±18.4	242.7±23.0	257.9±23.2
Pure tin spheres	12.3±2.2	27.3±3.5	52.6±5.3	76.6±7.8	111.2±8.6	128.6±8.6	153.6±15.7	175.6±12.3	214.7±9.6	245.1±10.7
Steel spheres	23.7±0.3	42.6±7.4	64.9±11.6	77.6±10.9	104.1±15.5	116.0±14.0	132.2±18.9	146.5±14.2	159.9±18.0	181.2±23.4

Values are the mean±1 S.D., based on five replicates per control. Units are mg metal/g sphere.

On days 1, 2, 3, 4, 7, 8, 9, 10, 11, and 14, a 1.0-ml sample of the medium was removed from every control and treatment replicate, and, after an appropriate dilution, was analyzed for copper, tin, and iron using ICPOES analysis. Each replicate sample was analyzed in 10% 1:1 hydrochloric acid:nitric acid. The ICPOES detection limits were set at 0.5 ppm for copper, 1 ppm for tin, and 5 ppm for iron. The wavelength was set at 324.7 nm for copper analyses, 189.925 nm for tin analyses, and 238.204 nm for iron analyses. A correction factor for the concentration of each metal was applied to adjust for 1 ml of solution that was removed from each previous treatment replicate. On day 14, the mass of the copper spheres, and the remaining tungsten–bronze material in each treatment bottle was measured.

2.1. *Statistical analysis of results*

Data analyses followed the general procedures outlined in Thomas et al. (2007a). The Shapiro–Wilk test was used to determine the normality of the distribution of the data set. Transformation of the data was not required. The effects of time and the presence of 0.6% iron in the tungsten–bronze shot on the dissolution of metals from the shot were determined by regression and repeated-measures analysis of variance, using a generalized linear model (GLM) framework and the SPSS (v12.0.1) statistical package (SPSS, 2003). Paired sample t-tests were used to assess the significance of the effect of sphere type on the dissolution of metals. Statistical significance was at the $P \leq 0.05$ level.

3. Results

3.1. *Dissolution of metals from the glass beads, tin, iron, and copper sphere controls*

There was no change in the mass of the glass bead controls over 14 days, and the levels of tin, and copper released from the glass beads were all below the limits of detectability.

The dissolution of metals indicated that the synthetic medium was capable of dissolving large amounts of copper, tin, and iron from the control material over 14 days (Table 3). A linear relation between time and copper dissolution occurred (Pearson correlation coefficient $r=0.907$; $P<0.001$; $y=86.39+14.16x$) and over the 14-day period, the mean rate of copper dissolution was 18.42 mg copper/g pellet/day. The control iron spheres were dissolved by the incubation medium, and a linear (Pearson correlation coefficient $r=0.992$; $P<0.001$; $y=21.80+12.03x$) release of iron (2.2 mg iron/g pellet/day) was observed across time (Table 3). The tin sphere controls released tin into the dissolution medium at a linear rate of 2.5 mg tin/g pellet/day, (Pearson correlation coefficient $r=0.993$; $P<0.001$; $y=-6.04+18.23x$) over the 14-day period (Table 3).

3.2. *Dissolution of metals from tungsten–bronze spheres*

The acid dissolution medium caused a rapid chemical break-up of the spheres used in the experiment, especially after day 6, and in the absence of any physical grinding action. All of the five spheres had disintegrated, completely, to fine powder by day 14 (Table 2). The spheres composed of tungsten and bronze (0% iron) lost an average of 21.3% of their initial mass after 14 days in the dissolution medium (Table 2). However, this loss was far less (21.3% versus 100%) ($P<0.001$) than that experienced by the tungsten–bronze spheres containing 0.6% iron (Table 2).

The tungsten–bronze spheres released copper, tin, and iron to the acid medium throughout the 14-day experimental period in a linear manner. For copper, the Pearson correlation coefficient $r=0.980$; $P<0.001$; $y=-34.50+37.79x$: for tin, $r=0.977$; $P<0.001$; $y=-4.47+3.39x$: for iron, $r=0.974$; $P<0.001$; $y=-0.487+0.495x$ (Table 4).

Both copper and tin were released from the tungsten–bronze (no iron) spheres in a linear manner over time. For copper, $r=0.989$; $P<0.001$; $y=19.95+6.16x$: for tin, $r=0.865$; $P<0.001$; $y=1.352+0.338x$. However, the absence of 0.6% iron in the tungsten–bronze formula caused a much slower

Table 4 – Dissolution of copper, tin, and iron from tungsten–bronze (0.6% iron) spheres over time

Treatment	Time, days									
	1	2	3	4	7	8	9	10	11	14
Copper	8.22±1.37	25.52±5.74	53.76±14.94	85.52±18.41	255.96±64.07	296.63±35.11	345.49±33.03	373.76±29.34	392.72±20.78	424.93±2.09
Tin	0.97±0.10	2.46±0.61	3.22±0.47	6.85±1.45	15.00±4.88	26.63±4.08	27.53±2.58	32.99±2.92	35.19±1.76	38.60±1.25
Iron	0.14±0.03	0.38±0.08	0.63±0.16	1.05±0.21	2.87±0.78	4.10±0.67	4.64±0.45	4.78±0.42	5.20±0.36	5.52±0.11

Units are mg metal/g sphere/day, ±1 S.D. There are five replicates for each mean value.

Table 5 – Dissolution of copper and tin from tungsten–bronze (0% iron) spheres over time

	Time, days									
	1	2	3	4	7	8	9	10	11	14
Copper	17.19±2.31	34.50±3.77	42.02±6.76	45.83±9.07	66.39±18.17	71.62±19.27	75.45±20.15	83.22±18.11	85.61±17.12	102.92±16.84
Tin	1.07±0.12	1.92±0.38	3.94±0.72	1.94±0.63	4.93±2.40	3.67±2.29	3.68±2.82	4.13±2.35	4.90±2.99	6.66±3.42

The units are mg metal/g sphere/day, ±1 S.D. Five replicates comprise each mean value.

dissolution of both tin and copper from the bronze component of the spheres (Table 5). By day 14 of the test, more than four times the amount of copper had been dissolved from the tungsten–bronze–0.6% iron spheres than the comparison without iron ($P<0.001$). The divergence in the copper dissolution rates was particularly marked after day 4 of the test, and continued to day 14. The inclusion of 0.6% iron had a similar effect on the dissolution rate of tin from the bronze component of the spheres (Table 4 and 5). At day 14 of the test, approximately six times more tin had been dissolved from the spheres containing iron than the spheres without iron ($P<0.001$). As with copper, the divergence in the dissolution rates was particularly marked after day 4 of the test.

3.3. *Amount of soluble copper, tin, and iron released, daily, from 8 tungsten–bronze spheres*

The average mass of one tungsten–bronze sphere of 3.48 mm diameter is 177.7 mg. It is assumed that 8 such spheres were present simultaneously in the hypothetical waterfowl. This figure is based on the number of ingested shot that, if made of lead, would induce certain acute mortality in waterfowl (USFWS, 1997). Eight spheres have a total mass of 1.42 g. The amount of copper dissolved across 14 days was 30.4 mg/g sphere/day: this is equivalent to 43.17 mg copper/day for 1.42 g of material. The tin dissolution was 2.74 mg/g sphere/day over the 14-day period, and is equivalent to 3.89 mg tin/day for 1.42 g of material. Iron was dissolved at the rate of 0.38 mg/g sphere/day, or 0.54 mg Fe for 1.42 g of material/day.

4. Discussion

Given that most of the tungsten–bronze spheres had disintegrated completely to metal powder and metals in solution before day 14 under chemical action, alone (Table 2), the break-up of the pellets would have occurred much more rapidly in a real waterfowl gizzard with additional physical grinding from grit. In this *in vitro* experiment, any small particles of bronze that are dislodged from the surface of the spheres were retained in the reaction vessel and continued to experience the dissolving action of the acid medium over 14 days. Such particles are likely to be small, 8–40 μm, reflecting the size of the metal components of the spheres (Thomas et al., 2007a). Particles of disintegrated spheres less than 1 mm in size would readily pass through the ventricular sphincter and enter the duodenum of a bird, and would escape subsequent acid dissolution in the small intestine. Thus the amount of dissolved copper, tin, and iron calculated to be theoretically available for uptake in the waterfowl bloodstream from this *in vitro* experiment may exceed the amount actually derived from an *in vivo* experiment.

4.1. *Influence of iron in spheres on the dissolution rates of copper and tin*

The presence of only 0.6% iron in the tungsten–bronze spheres enhanced the speed of fragmentation and the dissolution of both copper and tin dramatically. The likely explanation for this phenomenon is both chemical and physical. The iron is present as a solid solution constituent within the bronze material of the sphere. In a medium of pH 2.0, iron dissolved at a greater rate than either copper or tin. As a consequence, over a period of time the surface area of the pellet increases as iron is removed which in turn increases the dissolution of copper and tin. Thus the data in Tables 2, 4 and 5, reveal a positive interaction between time and increasing surface area of the pellet. The dissolution profiles for copper and tin are very similar, suggesting, again, that the increased rate of dissolution of these metals over time is an artifact of increased surface area of the pellet, and not some chemical property of copper and tin in the pellet that caused increased solution over time.

4.2. *Potential toxicity of 8 ingested tungsten–bronze spheres to birds*

The potential toxicity of the copper, iron, and tin dissolved from eight spheres ingested simultaneously is considered, especially to birds likely to encounter discharged shot. Assume further that the waterfowl are ingesting 150 g of dry food each day. Any discussion of metal toxicity has to be aware of potential interactions among metals at the level of the gut, and also reflect the changing seasonal physiology and metal requirements of the birds, besides species-related metal requirements and/or toxicities (Cork, 2000).

4.2.1. *Iron*

The daily iron intake recommended for adult geese and pheasants (*Colchicus* spp.) is 50–80 mg/kg feed, depending on their reproductive condition (Leeson and Summers, 1997). Commercial ducks require 46–80 mg/kg feed, depending on their breeding condition (Leeson and Summers, 1997). Using the lower requirement figures, this equates to 6.9–12.0 mg iron in 150 g of feed consumed daily. Thus the 0.54 mg of iron released daily from 8 ingested spheres is far below the nutritional requirements of these two species. Iron shot has been legally-approved as a lead shot substitute by the USA since 1991 (USFWS, 2006), and iron shot controls have been used frequently in the testing of the acute and chronic toxicity of various lead substitutes on captive waterfowl (e.g. Grandy et al., 1968; Sanderson et al., 1997; Mitchell et al., 2001a,b,c). These studies reported that the continuous presence (verified radiographically) of iron shot in the gizzard was not accompanied by negative impacts on reproduction, blood parameters, and the general health of ducks. Thus the release of iron into the bloodstream of ducks from eight ingested tungsten–bronze spheres will not induce toxic effects.

4.2.2. *Tin*

Tin is not a required element in the diet of birds and mammals. Consequently, few studies exist in which elemental tin has been administered to birds at varying levels. However, the potential use of tin as a lead substitute has generated studies in which tin, either as a pure metal, or present as an alloy with other metals, has been administered to captive waterfowl, and the effects on the birds' health monitored. In a 30-day acute toxicity test on captive mallard ducks, eight pure tin shots were placed in the gizzard and the birds were later necropsied (Grandy et al., 1968). No signs of a toxic effect of tin were observed in any of the variables measured. In a similar, chronic, assessment of the potential toxicity of tin, Gallagher et al. (2000) administered eight pure tin shots to adult mallard ducks over 150 days, and measured the effects of tin on blood parameters, metabolites, tissue deposition, and reproductive performance, as well as effects on developing ducklings. All the tin-dosed ducks survived and reproduced normally, as did the progeny, and no toxic effects were detected for any variable, including tissue pathologies.

In view of the results of the previous two studies using 100% tin, and given that tungsten–bronze spheres comprise only 3.9% tin, by mass (Table 1), it is unlikely that the release of tin from ingested spheres will cause acute or chronic toxic effects upon the physiology and health of birds.

4.2.3. *Copper*

Eight tungsten–bronze spheres will liberate 43.17 mg copper/day, over 14 days of retention. This is meant to be a worst-case scenario. Assume further that the molybdenum status of the bird is adequate, and not liable to complicate copper metabolism. Then 43.17 mg copper/day is the same as 287.8 ppm of the daily diet. Leeson and Summers (1997) include copper in the diet of commercial geese and ducks at 4–8 mg/kg diet (or 0.6–1.2 mg/150 g feed/day), depending on breeding activity, a level below the 43.17 mg of copper released from the spheres. This level of copper is now compared with copper levels known to cause no effects, or be toxic, to birds in the published literature. There is a metabolic interaction among dietary copper, iron, molybdenum, and zinc at the level of small intestine that influences bioavailability, potential absorption, and potential toxicity (Davis and Mertz, 1987). Thus, the levels of copper that are released from the spheres may be moderated in the gut. However, this analysis presents only those copper levels actually released in the *in vitro* experiment.

Irby et al. (1967) dosed 24 mallard ducks with eight pure copper shots (total mass 0.6 g) to observe if this lead substitute was toxic during a 60-day exposure. In the present, *in vitro*, simulated gizzard test, the amount of copper in eight tungsten–bronze spheres is 44.4% of 1.42 g, or 0.63 g. This is very comparable to the study of Irby et al. (1967). Irby et al. (1967) reported that none of the copper-dosed ducks died from copper toxicosis after 60 days. If the copper sphere control used in the present *in vitro* experiment can be compared to the *in vivo* conditions inside live ducks, then 0.6 g of copper shot would release 11.05 mg of copper for each of 60 days (based on copper sphere controls dissolving at 18.42 mg/g copper/day). The observed rate of copper release from 8 tungsten–bronze spheres is higher than this value (43.17 mg versus 11.05 mg Cu/day).

However, in the Irby et al. (1967) study, the ducks were exposed to this level for 60 days. In the event of ducks ingesting tungsten–bronze spheres, the very rapid break-up of the pellets will result in none being in the gizzard for that long a period. Chemical dissolution, alone, would result in a residence time of, at most, 14 days. The grinding action of the gizzard assisted by grit would cause complete fragmentation in a much shorter time, probably less than 1 week. Moreover, the fine pieces of tungsten–bronze that are released in a gizzard would quickly leave the gizzard, so lowering the overall dissolution of copper. In this simulated *in vitro* experiment, such fine pieces remained in the dissolution medium, and yielded more copper. Thus the theoretical availability of copper from this test must be considered maximal when comparing it to the Irby et al. (1967) study. What can be safely concluded is that when ducks ingest 1.42 g of tungsten–bronze spheres, at most 43.17 mg of copper will be released each day for about 1 week or less.

Henderson and Winterfield (1975) reported a case of acute copper toxicity in three-week-old Canada geese (*Branta canadensis*) that had ingested water contaminated with copper sulfate. These authors calculated the copper intake to be about 600 mg copper sulfate/kg body weight, or 239 mg Cu/kg. The amount of copper released from 8 spheres is 43.17 mg Cu, a figure far below the 239 mg Cu/kg goose required to produce acute toxicity, even in a growing gosling.

Young (8-day-old) commercial ducklings were fed a diet supplemented with 100 ppm of copper as copper sulfate for 8 weeks (King, 1975). This author reported a promotion of growth compared to controls at the end of 2 months, but some thinning of the caecal walls. Jensen et al. (1991) conducted performance tests over 3 weeks on growing day-old chickens whose diets contained varying levels of added soluble copper. These authors concluded that the maximal improvement in live body weight gain, and feed conversion efficiency, occurred at dietary levels of copper of 169 ppm and 140 ppm, respectively. Mehring et al. (1960) listed a figure of 500 ppm as the minimal toxic level of dietary copper for growing chickens. Poupoulis and Jensen (1976), in a similar set of four-week performance tests with day-old chickens and copper additives to the diet, reported that no gizzard lining erosion could be detected in chicks fed 125 ppm of copper, but some slight erosion of the gizzard lining occurred in chicks fed 250 ppm copper. Note that this result was seen in very young birds over a one-month period of growth. It required 500 to 1000 ppm of copper to depress growth and body weight gain of chicks in this experiment (Poupoulis and Jensen, 1976).

The influence of dietary copper addition on the body mass and reproduction of mature domestic chickens was analyzed by Stevenson and Jackson (1980). Hens fed on a diet containing 250 ppm copper for 48 days showed a similar daily rate of food intake as control hens (0-copper in the diet). The mean number of eggs laid daily also did not differ between hens fed 250 ppm copper and controls. Negative effects on the daily food intake, body mass loss and egg laying rates were observed only at dietary copper levels in excess of 500 ppm, and after 4 months of being fed such diets.

Similar performance tests on growing domestic turkeys have been published, and can be used as a further species comparator. Copper at the level of 300 ppm in the daily diet produced no long-term effect on growing (1-week-old) turkey poults, but 800 ppm of copper in the diet for 3 weeks inhibited growth (Supplee, 1964). Vohra and Kratzer (1968) reported no effect of feeding 400 ppm of copper as copper sulfate to turkey poults in the daily diet for 21 weeks, and concluded that poults could tolerate 676 ppm of copper without exhibiting deleterious effects. However, these authors reported reduced growth of poults fed 800 ppm and 910 ppm of copper over the same time, and death at 3240 ppm in the diet. This conclusion was supported by Christmas and Harms (1979), who found that copper in the diet of domestic turkeys had to rise to the 500–750 ppm level before signs of slight toxicity appeared, assuming that adequate methionine were also present.

Given that 43.17 mg copper released from tungsten–bronze spheres per day in a diet of 150 g of dry food is equivalent to 287.8 ppm copper, the release of copper from the eight ingested tungsten–bronze spheres would not present a toxic threat to laying fowl, or growing fowl. A copper level of 288 ppm in the diet is below the level of 500 ppm required to produce toxic syndromes in fowl. Haseltine (1986) reported that no signs of copper toxicity attended 250 ppm copper in the diet of mallard ducks for several weeks, suggesting that short-term exposure to lower doses of copper would not be met with mortality nor reproductive impacts. The Committee on Mineral Toxicity in Animals (1980) concluded that the maximum tolerable levels of dietary copper during the long-term growth of chickens and turkeys should be 300 ppm. This recognizes that species susceptibility to copper may exist related to differences in the dietary and tissue status of iron, molybdenum, zinc, and sulfur (Underwood, 1971).

The tungsten–bronze–0.6% iron spheres will also liberate iron ions at the same time that copper is being dissolved in the gizzard (Table 6). The iron in solution could moderate the uptake of copper from the small intestine of the bird (see Davis and Mertz, 1987). In a study on young pigs, Suttle and Mills (1966) reported that the adverse effects of feeding copper at the rate of 250 ppm and higher were prevented by feeding slight increases in the daily levels of iron and zinc. Assuming that the same situation applies to fowl and other wild birds, the presence of iron in the tungsten–bronze shot could lower, further, the apparent presence of dissolved copper in the gut.

4.2.4. *Tungsten*

Soluble tungsten salts in high concentrations have been identified as being toxic to components of soil biota (Strigul et al., 2005), and others have construed this finding to imply that elemental tungsten in spent gunshot is also toxic to the general environment, including birds, when ingested (Ogundipe et al., 2007). Thomas et al. (2007b), in a response to Ogundipe et al. (2007), indicated that for that scenario to occur, levels of spent tungsten-based shot would have to exceed U.S. Fish and Wildlife Service criteria for maximal shot deposition many times.

The published studies on the potential toxicity of ingested elemental tungsten most relevant to the present experiment are those of Mitchell et al. (2001a,b,c), in which the physiological effects of ingested tungsten–iron shot were measured in mallard ducks after 150 days' chronic exposure. The composition of the alloyed shot was 55% tungsten and 45% iron, similar to the composition of the tungsten–bronze spheres used in the present experiment.

Mitchell et al. (2001a) reported that the continuous presence of tungsten–iron shot in the gizzard of ducks over 150 days was not accompanied by any adverse effects upon survivability, the mass of the body, spleen and gizzard, and the cytological structure of the liver and kidneys. Tungsten was released from the tungsten–iron shot during this time, their losing about 28% of the initial mass in the gizzard. Solubilized tungsten entered the body of the ducks, but was not reported to have been accompanied by toxic effects upon the adults, their reproduction or their progeny. The hematocrit, hemoglobin content, and whole-blood delta aminolevulinic acid dehydratase (ALAD) activity in adult ducks given tungsten–iron shot were statistically similar to controls after 150 days (Mitchell et al., 2001b). The level of plasma uric acid, and the activities of plasma alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, and lactate dehydrogenase in the same ducks showed no statistically significant effect of tungsten absorption from the administered shot. Tungsten was present in the femurs, kidneys, and liver of the adult ducks (Mitchell et al., 2001b, Tables 4–7), but was not associated with signs of toxicity.

In Mitchell et al. (2001c), the transfer of tungsten from the adult females dosed with tungsten–iron shot for 150 days to eggs and ducklings was measured. The presence of tungsten in the body of the adult female had no measurable effects upon the percentage egg production and the fertility and hatchability of eggs. Tungsten was detected in both the shell and whole contents of eggs, but at levels close to the detection limits. The femurs, kidneys and livers of ducklings contained tungsten, but again, at levels close to the experimental detection limits (Mitchell et al., 2001c, Tables 3–5). Ducklings derived from mallard hens given the tungsten–iron shot had the same survivability, body mass, and blood hematocrit as their controls.

In an older, but related, study Teekell and Watts (1959) fed soluble sodium tungstate at the level of 250 and 500 ppm in the diet to laying hens, but reported no influence on the egg laying or the hatchability of the eggs. Both of the levels would certainly exceed the rate at which tungsten could be removed and solubilized from the tungsten–iron shot during their stay in the gut, based on the approximate rate at which tungsten–iron shot lost mass over 150 days in the gut of ducks (Mitchell et al., 2001a). In the present study, tungsten–bronze spheres containing 0.6% iron disintegrated rapidly in the digestion medium, and would have passed through the gut well before 14 days (Table 2), so minimizing the time during which tungsten would have been solubilized and absorbed.

Collectively, the three studies of Mitchell et al. (2001a,b,c) indicate that tungsten as tungsten–iron alloy shot present in adult ducks over 150 days did not affect adversely the adult birds, their reproduction, and the viability of their progeny. Given the similar proportion of tungsten in the tungsten–iron and tungsten–bronze shot, it is possible that any tungsten liberated from ingested tungsten–bronze spheres *in vivo* might produce results similar to those reported in Mitchell et al. (2001a,b,c), especially in view of their rapid rate of disintegration. Furthermore, no synergistic, physiologically-adverse effects between tungsten and copper, tungsten and tin, and their combinations have yet been described.

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