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Review

Assessment of the environmental toxicity and carcinogenicity of tungsten-based shot☆,☆☆

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ABSTRACT

The toxicity of elemental tungsten released from discharged shot was assessed against previous studies that established a 1% toxic threshold for soil organisms. Extremely heavy theoretical shot loadings of 69,000 shot/ha were used to generate estimated environmental concentrations (EEC) for two brands of tungsten-based shot containing 51% and 95% tungsten. The corresponding tungsten EEC values were 6.5–13.5 mg W/kg soil, far below the 1% toxic threshold. The same shot loading in water produced tungsten EEC values of 2.1–4.4 mg W/L, levels that are not toxic under experimental conditions. Pure tungsten has not been shown to exhibit carcinogenic properties when ingested or embedded in animal tissues, but nickel, with which it is often alloyed, has known carcinogenicity. Given the large number of waterfowl that carry shot embedded in their body, it is advisable to screen lead shot substitutes for their carcinogenic potential through intra-muscular implantation.

Contents

1. Introduction

The large body of evidence implicating spent lead gunshot in the primary lead poisoning of waterfowl and the secondary lead poisoning of birds of prey (US Fish and Wildlife Service (USFWS), 1986; Fisher et al., 2006) has led to a rapid development of lead substitutes, especially since 1991 when the USA and Norway banned the use of lead shot for hunting waterfowl (Beintema, 2001). To date, lead substitutes made from iron, tin, bismuth–tin, tungsten–plastics, tungsten–bronze, tungsten–nickel–iron, tungsten–iron, and other mixtures of these metals have been developed and approved for legal use in the USA and Canada (US Fish and Wildlife Service (USFWS), 2006). The utility of tungsten in these forms of shot relates to its high density (19.35 g/cm^3) and the need to develop substitutes that approach the ballistic characteristics of lead. The Canadian and US legal processes

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for regulating the composition of new substitutes require that empirical evidence be generated from controlled toxicity tests indicating that shot ingested by waterfowl do not pose a toxic threat to the birds (US Fish and Wildlife Service (USFWS), 1997). The need to demonstrate that a new candidate shot is non-toxic to other life forms in the environment is less stringent (Thomas and Guitart, 2003). Nations other than Canada and the USA lack any legal mechanisms to control the composition of lead substitutes (Thomas and Guitart, 2003), whether used as gunshot or fishing weights. The legal approval of tungsten as a non-toxic component of gunshot is based mainly on avian studies conducted over at least 150 days and across two generations under conditions that would demonstrate pathologies and diverse toxic signs, were the material toxic (US Fish and Wildlife Service (USFWS), 1997). Recently, based on studies of soil organisms, concerns have been raised about the toxicity of tungsten and certain tungsten alloys to components of the environment other than waterfowl (Begley, 2004; Dermatas et al., 2004; Strigul et al., 2005; Koutsospyros et al., 2006). Ogundipe et al. (2007) used these concerns as a basis for questioning the use of tungsten-based shot. Kalinich et al. (2005) implicated a tungsten alloy in the generation of malignant tumours when implanted intra-muscularly in F344 rats. It has also been suggested that tungsten of geological origin might be involved in the cluster of childhood leukemias at Fallon, Nevada (Centres for Disease Control (CDC), 2003).

It has only been during the past decade that release of elemental tungsten to the aquatic and terrestrial environment through game shooting has taken place, and independent, detailed studies of the environmental effects of tungsten are few compared to those of other heavy metals such as cadmium and mercury. The purpose of this paper is to assess the risk posed by spent tungsten shot to organisms other than waterfowl and to investigate whether the putative carcinogenicity of tungsten is due to tungsten *per se*, or other metals that tungsten may be combined with in shot. Also, by comparing the amount of tungsten that would be released to the environment under the most extremely heavy shooting conditions with levels of tungsten that Strigul et al. (2005) regarded as toxic, we can begin to assess the environmental risk posed by spent tungsten-based shot.

2. Establishing the environmental conditions as the basis for comparison

The regulations applied by the US Fish and Wildlife Service (US Fish and Wildlife Service (USFWS), 1997) established a 'worst-case scenario' for assessing the potential toxicity of a candidate non-toxic (non-lead) shot. In this, it is assumed that 69,000 shot of No. 4 size (3.07 mm diameter) will be dispersed over 1 ha of soil to a depth of 5 cm, or over 1 ha of water to a depth of 30.48 cm. Then, based on the percentage of tungsten (by mass) in the shot material, the estimated environmental concentration (EEC) of tungsten can be calculated as the amount of tungsten in 500 m^3 of soil, or 3048 m^3 of water. The EEC value assumes that all of the tungsten in the shot has been solubilized, that adsorption of tungsten onto organic and inorganic fractions in both soil and water has not occurred, and that all of the tungsten is available biologically. The figure of 69,000 shot per hectare is based on known densities of spent shot in the most heavily shot-over regions of the USA. It represents a theoretical shot density meant to challenge the potential non-toxicity of any lead shot substitute. Since this scenario can be applied to shooting situations world-wide, it is the basis of the present comparison.

Two commercial tungsten-based shot types were used for the comparison. Tungsten–matrix shot comprises 95% tungsten by mass and tungsten–bronze shot comprises 51% tungsten by mass.

Table 1
Chemical composition of shot types containing tungsten approved as non-toxic for hunting waterfowl in the US and Canada.

Approved shot	Shot composition, by mass
Tungsten–iron	Any proportion of W and ⩾1% Fe
Tungsten–iron–nickel	Any proportion of W, ⩾1% Fe, and up to 40% Ni
Tungsten–iron–tin	Any proportions of W and Sn, and ⩾1% Fe
Tungsten–iron–copper–nickel	40–76% W, 10–37% Fe, 9–16% Cu, 5–7% Ni
Tungsten–iron–tin–nickel	65% W, 10.4% Fe, 21.8% Sn, 2.8% Ni
Tungsten–bronze (2 products)	51.1% W, 44.4% Cu, 3.9% Sn, 0.6% Fe
Tungsten–bronze	60% W, 35.1% Cu, 3.9% Sn, 1% Fe
Tungsten–tin–bismuth	Any proportions of W, Sn, and Bi
Tungsten–matrix	95.9% W, 4.1% polymer
Tungsten–polymer	95.5% W, 4.5% Nylon 6 or 11

Table contents are based on data in US Fish and Wildlife Service (USFWS) (2006). Shot coatings of copper, nickel, tin, zinc, zinc chloride, and zinc-chrome are also approved for use on approved types of non-toxic shot. Not all of the shot types listed below may be sold widely in North America.

Both brands of cartridge are sold widely in North America, and they represent the upper and lower levels of elemental tungsten in the non-toxic shot formulations (Table 1). Given the mass of a single No. 4 tungsten–matrix shot as 213 mg, the calculated tungsten EEC for water is 4.44 mg/L and, for soil, is 13.54 mg/kg, where soil has a mass of 2 kg/L. The mass of 1 pellet of tungsten–bronze shot is 183.2 mg: the tungsten EEC for water is 2.12 mg/L and, for soil, 6.46 mg/kg.

The EEC for tungsten in other brands of tungsten-based shot can be calculated from their gross composition (Table 1). Similarly, the tungsten EEC in soils of density other than 2.0 can readily be calculated, arithmetically.

3. Comparison of EEC values with other studies reporting tungsten toxicity

Strigul et al. (2005) reported that tungsten powders incorporated into soil at levels exceeding 1% by mass induced changes in the soil community, such as death of bacteria and an increase in the fungal population. The same study indicated that the degradation of starch applied to soil was inhibited completely when the soil contained more than 3% tungsten by mass. This study also observed the effects of tungsten powders in soils on the survivability of earthworms, concluding that all the worms survived 14 days of exposure to 10–1000 mg tungsten/kg soil.

Studying the effects of tungsten on the survivability of soil bacteria, Strigul et al. (2005) reported that, after 3 months, 95% of bacteria had died following exposure to soils containing 3% tungsten by mass. However, when highway soils were treated with tungsten on a 1% and 0.01% mass basis, no significant toxic effects were observed at the 0.01% concentration (i.e. 100 mg tungsten/kg soil) after 1 year. Strigul et al. (2005) also reported that ryegrass germinated in soils containing 10% by mass tungsten died after 1 month. A threshold level of soil tungsten was identified as 0.1–1% by mass for inhibition of ryegrass growth. The authors concluded from these results that elemental tungsten in soils could have detrimental environmental effects above a threshold level of 1%. In studies on solubility, sorption, and soil respiration of tungsten and tungsten alloys, Dermatas et al. (2004) reported that elemental tungsten added to soils above 3% by mass adversely affected the respiration of soil microbes.

As previously detailed, the tungsten EEC levels for tungsten–matrix shot and tungsten–bronze shot in a 'worst-case' scenario with the heaviest shot loading circumstances are 13.54 mg/L, and 6.46 mg/kg soil, respectively. These two values—equivalent to

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0.0014% and 0.0006%, respectively—are far below the 1% and 3% by mass threshold levels that Strigul et al. (2005) and Dermatas et al. (2004) identified as being toxic to soil communities.

Ogundipe et al. (2007) cited a study by Tajima (2003) to demonstrate the potential toxicity of tungsten. However, Tajima (2003) concluded that, based on the influence of soluble tungsten salts on the activity of the umuDc gene in *Escherichia coli*, tungsten salts were both biologically and toxicologically inert. Tajima indicated that soluble tungsten salts have biological effects on *E. coli*, but did not equate these to toxicity. Sugio et al. (2001) investigated the mechanism of the inhibition of growth of *Acidithiobacillus ferooxidans* by sodium tungstate and observed that growth was inhibited in media where the salt concentration was 14.7 mg/L. This level is higher than the 'worst-case' tungsten EEC for both types of shot under consideration.

Under circumstances where tungsten becomes solubilized, there is a potential for tungsten salts to become adsorbed onto organic and mineral components of both soil and water. Dermatas et al. (2004) reported that soil fractions readily adsorb tungstate salts in a non-reversible manner. Presumably, this would lower the soil EEC values for tungsten, depending on the degree of adsorption and affirm further the non-toxicity of spent tungsten-based shot to soil organisms.

It is informative to relate the 'worst-case' scenario tungsten EEC values to naturally occurring levels of tungsten in the environment. Senesi et al. (1988) measured the level of naturally occurring tungsten in an array of soils and reported background levels between 0.2 and 2.4 mg/L soil. Extrapolating from these levels, the heaviest tungsten soil loading from spent shot at the most heavily shot-over sites would be, at most, five times the highest background level. Quin and Brooks (1972a) measured tungsten in the soils around agricultural lands in New Zealand, reporting levels of 1.9–21.4 mg W/kg soil. However, in areas where the soils were heavily mineralized, tungsten levels were much higher, ranging from 65 to 125 mg W/kg (Quin and Brooks, 1972b). The 'worst-case' tungsten EECs for both brands of tungsten shot fall far below these levels. The federal governments of Canada and the US Environmental Protection Agency do not have standards for tungsten in sludges or biosolids applied to soils (see US Environmental Protection Agency (USEPA), 1995).

4. Potential toxicity of soluble tungsten in drinking water

The USA, Canada, and the Member States of the European Union do not have potable water standards for tungsten. A number of independent studies have investigated the effects of ingested soluble forms of tungsten in drinking water on different physiological parameters. Rats given sodium tungstate at 200 mg/L for 20 weeks did not exhibit changes in body weight or any notable histopathology (Luo et al., 1983). Giving rats drinking water containing 100 mg sodium tungstate/L for 3 weeks produced no effects on bodyweight or liver weight, nor effects on succinate-cytochrome *c* reductase[1] activity (Cohen et al., 1973). Munoz et al. (2001) reported no deleterious effects on growth or on the liver and kidney of rats given drinking water containing 2000 mg sodium tungstate/L for 2 months. Schroeder and Mitchener (1975) reported that rats given tungsten at 5 mg/L in their drinking water for their entire life showed a slight increase in growth and a slight reduction in longevity.

Given that the 'worst-case' aquatic tungsten EECs for tungsten–matrix shot and tungsten–bronze shot are 4.44 and 2.12 mg W/L, respectively, the above-cited studies on the effects on rats of drinking water containing much higher levels of sodium tungstate indicate that concerns relating to the possible toxicity of animals' drinking water containing soluble tungsten derived from spent shot are unwarranted.

5. Implicating tungsten in carcinogenicity

While this review deals primarily with the fate of elemental tungsten in gunshot, tungsten compounds of geological origin and other anthropogenic origins can enter the human environment and the human food chain through potable water and other ingesta. Concerns about a potential carcinogenic role of tungsten have arisen from tungsten compounds in drinking water (Centres for Disease Control (CDC), 2003) and the use of tungsten in ballistic heavy metal alloys (Kalinich et al., 2005) and have been used to question the presence of tungsten in new types of gunshot.

5.1. Carcinogenicity associated with tungsten in potable water

The deposition of gunshot in water bodies and wetlands by intense hunting pressure across years raises the possibility that tungsten could become mobilized from the shot and become part of human potable water. In situations where such gunshot undergoes slow disintegration on dry land, small particles of tungsten, either as metal or tungsten compounds, could reach humans and be inhaled or ingested. Kalinich (2005), Koutsospyros et al. (2006), and Ogundipe et al. (2007) referred to a possible relationship between tungsten in the environment of humans in Fallon, Nevada, USA and certain types of leukemia in children. This occurrence of leukemia in children has been examined in detail as to its possible cause(s), including (but not confined to) the presence of elevated levels of soluble tungsten in the potable groundwater. Seiler et al. (2005) identified elevated levels of tungsten in ground water around Carson Desert, Nevada, and attributed these levels to the natural erosion of tungsten bearing minerals in the local watershed, possibly reinforced by upwelling from deep warm waters. Sheppard et al. (2006) measured both tungsten and cobalt levels in atmospheric particles from the Fallon, Nevada region, and suggested that they originated from a hard-metal processing plant in Nevada. Whatever the origin (natural and/or anthropogenic) and form of the tungsten in the human environment, mention and examination of its potential carcinogenicity is warranted.

The Centers for Disease Control and Prevention concluded that while tungsten was "*a potentially unique exposure within Churchill County*" [i.e. Fallon], it was not identified as the cause of the leukemia (Centres for Disease Control (CDC), 2003) and this Agency could not detect a statistically significant relationship between exposure to ingested tungsten in drinking water and childhood leukemia in Churchill County, Nevada (Centres for Disease Control (CDC), 2003). The Expert Panel on Childhood Leukemia in Churchill County, Nevada (Expert Panel on Childhood Leukemia, 2004) concluded that tungsten had likely been present in that environment for many years (from mining, a tungsten smelter and use of tungsten ammunition at a nearby military base) and could not link tungsten in the human environment to leukemia in children. Furthermore, three major agencies, the US Department of Health and Human Services, the US Environmental Protection Agency and the International Agency for research on Cancer, have not linked tungsten exposure with carcinogenic effects. However, the US National Toxicology Program has been advised to investigate further all the potential effects of tungsten on animal health (ATSDR (Agency for Toxic Substances and Disease Registry), 2005a, 2005b).

[1] A mitochondrial respiratory enzyme.

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Daughton (2005) suggested that the actual cause(s) of the leukemia remained to be identified and hypothesized that a range of other environmental agents could contribute to carcinogenicity. Rubin et al. (2007) re-evaluated the potential environmental causes of the childhood leukemia in Churchill County, paying special attention to tungsten exposure. These authors could not establish, scientifically, any link between tungsten and leukemia and indicated that the elevated tungsten levels in Churchill County were not unique compared to adjacent regions in which exceptional incidences of leukemia did not occur. However, a recent study by Sheppard et al. (2007) used dendrochemistry to monitor airborne metals in the environment around Fallon, Nevada. Cottonwood (*Populus* sp.) trees revealed an increase in tungsten levels from the mid-1990s, and increased cobalt levels from an earlier time, but no temporal increases were seen in other metals. The authors recommended that the potential roles of tungsten and cobalt, in combination, in the generation of tumours be investigated further, a recommendation made also by Sheppard et al. (2006).

5.2. Carcinogenicity associated with ingested and muscle-embedded tungsten-based shot

Tungsten-based shot can enter the body of animals and humans in several ways. They can be ingested directly as spent shot; they can enter the digestive tract when the tissues of animals killed with tungsten-based ammunition are eaten, as in the case of predators and humans, and the shot may enter the body from non-lethal gunfire and be carried in tissues. The presence of shot in the body can have various toxicological consequences, including acute toxicity, chronic inflammation and carcinogenicity, with different physiological circumstances determining the residency, solubility, excretion, and potential toxicity of shot materials in the gut versus shot embedded in muscle.[2]

It is necessary to consider the variety of metals that may be combined with tungsten in different brands of commercial shot and the manner in which they are combined, since this may determine their bio-availability. Thus tungsten–bronze shot is a sintered mixture of bronze powder and tungsten powder (Thomas et al., 2007), whereas tungsten–nickel–iron shot is a true alloy of these three metals. The physico-chemical interactions among metals in true alloys or sintered mixtures determines how quickly individual metals can be solubilized and exert their influence (Ogundipe et al., 2006).

Ringelman et al. (1993), Kelly et al. (1998), Mitchell et al. (2001a, 2001b, 2001c), and Brewer et al. (2003) have shown that elemental tungsten, whether combined with plastics or sintered or alloyed with other metals, does not pose a toxic threat to captive waterfowl when ingested. It is upon these controlled studies that full, unconditional, approval of tungsten-based shot has been given by the USA and Canada.[3] These studies required histopathological examination of the principal organs of mallard ducks to be examined by certified pathologists. Should any tumours have developed within the 30 or 150 day period, legal approval would not have been given. In a separate study, tungsten–bismuth–tin shot, when embedded in muscles of mallard ducks for 8 weeks, did not produce any adverse or toxic effects (Kraabel et al., 1996). To date, only the study by Kraabel et al. (1996) has investigated the effect of embedding tungsten-based shot intra-muscularly in the birds: all the other studies were performed with tungsten-based shot present in the digestive system.

In the study by Kalinich et al. (2005), tungsten–nickel–cobalt alloy pellets (W 91.1%: Ni 6.0%: Co 2.9%) implanted into the muscle of F344 rats induced potentially fatal malignant tumours, indicating that tungsten alloys are carcinogenic by this exposure route, a point raised also by Koutsospyros et al. (2006). Similar pellets made from nickel also produced tumours, but a tantalum control did not. Unfortunately, the Kalinich et al. (2005) study did not contain a pure tungsten control and so it is not possible to determine the role, if any, played by tungsten itself in the generation of the tumours. The same caveat was noted by ATSDR (Agency for Toxic Substances and Disease Registry) (2005a). Kalinich et al. (2005) did suggest a possible combined effect of all three metals and specifically alluded to possible evidence for synergism between nickel and cobalt.

Several independent studies have investigated the long-term effects of pure tungsten coil implants, focusing on *in vivo* corrosion of the metal and any associated toxicity. Peuster et al. (2003a, 2003b) implanted tungsten coil sutures in rabbit and human tissues and examined their fate and possible toxicity. Peuster et al. (2003a) concluded that while there was mobilization of tungsten from the suture coils implanted into humans, the rate of mobilization was very low (29 μg/day). The results indicated no toxic effects in human adult and pediatric patients despite elevated serum tungsten levels. In their 2003b study, Peuster et al. implanted tungsten coils into the subclavian artery of rabbits and observed the effects 4 months later. The authors reported an increase in serum tungsten levels from 0.48 μg/L before implantation to 12.4 μg/L 4 months after implantation. However, the dissolution of tungsten from the coils was not accompanied by any local or systemic toxicity. Corrosion of pure tungsten implants in humans and accompanying elevated blood tungsten levels has also been reported by Butler et al. (2000) and Barrett et al. (2000). However, both studies did not report toxic effects in patients many months after implantation. This line of research was continued by Bachthaler et al. (2004) in which pure tungsten implants were monitored in human patients over several years. These authors did not observe toxic effects in any patient with elevated blood tungsten levels. However, Bachthaler et al. (2004) did caution against the use of such implants because superior materials were available that did not undergo corrosion, and because the clinical significance (if any) of elevated tissue tungsten levels remained to be determined.

Thus there is no direct evidence that pure, elemental tungsten causes toxicity or carcinogenicity. Leggett (1997) developed a model to infer more about the distribution and retention of tungsten in the human body and stated that while the data on this subject are…"*weak and inconclusive, the occupational experiences and the available toxicological studies on laboratory animals suggest that tungsten may have a relatively low order of chemical toxicity.*" van der Voet et al. (2007) did not identify any specific adverse effects attributable to tungsten in a review of this metal's clinical properties. However, these authors stated, explicitly, the need to distinguish between elemental tungsten and other heavy metals with which it is normally alloyed in inducing tumours, and cited nickel and cobalt, specifically, as contributors to such risk. van der Voet et al. (2007) reiterated the precautionary remarks of Butler et al. (2000) and Bachthaler et al. (2004) about elevated levels of tungsten in human tissues and the need for more research, both on the toxic risks posed by the pure metal in the body, and the carcinogenic risks posed by other metals' presence in tungsten alloys.

[2] *Note*: Although testing of the (non)toxicity of some new types of shot by implanting them into the muscles of ducks has been conducted, it is not a legal requirement of the US or Canadian regulations.

[3] Approval can be revoked should toxicity issues or other environmental problems arise during use of the new shot.

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5.2.1. Nickel in tungsten alloys: inflammation and carcinogenicity

Hoots et al. (2007) implanted shot made from nickel-coated steel, tungsten–polymer, tungsten–iron, and tungsten–nickel iron into the musculature of rats and observed the local and systemic effects 26 weeks later. They found that the three tungsten-based shot types produced no neoplasms after 26 weeks. Nickel-coated steel shot underwent a significantly greater corrosion than the other shot types and produced a marked local tissue inflammation 3 weeks after implantation, but not after 26 weeks. Severe inflammatory reactions in rabbit muscle to implants of nickel–cobalt alloys were also reported by Laing et al. (1967) and in rat muscle from implants of pure nickel and cobalt by McNamara and Williams (1981). Uo et al. (2001) reported that nickel implants in rat muscles caused marked tissue damage at the sites of implantation and demonstrated that nickel had the highest relative metal toxicity of all the metals tested. Thus there is strong evidence for the inflammatory effects of implanted nickel, but not pure tungsten.

Several independent reviews have established nickel and nickel compounds as carcinogenic. The National Toxicology Program (2005) found a number of studies that revealed the carcinogenic nature of nickel compounds, related to the slow release of nickel ions that exert a genotoxic effect throughout the body. The review of Kasprzak et al. (2003) cites evidence for the genotoxic and mutagenic activity of nickel ions, especially at higher tissue levels. Salnikow and Kasprzak (2005) indicated that a major prerequisite for nickel toxicity is prolonged action at the tissue site, as might occur from the implantation of metallic nickel into muscle.

Miller et al. (2000) showed that metallic nickel causes neoplastic transformation in cultured cells. Miller et al. (2001, 2004) subsequently attempted to differentiate the potential toxic effects of elemental tungsten, nickel, and cobalt that are the principal component of military penetrators. In the 2001 *in vitro* study on human osteoblast cells, Miller et al. measured a decrease in cell survival after 5 weeks exposure to tungsten, nickel and cobalt powders in a dose-dependent manner. However the neoplastic transformation of osteoblasts was far greater when cells were exposed to the tungsten alloys. In a further experiment, Miller et al. (2004) observed dose-dependent activation of 13 gene promoters by tungsten, nickel, and cobalt, alone, but the effect was statistically significant only at the highest dose levels. The genes induced are related to DNA damage and the development of malignancy (Miller et al., 2004). As with the Miller et al. (2001) study, the level of gene induction by each metal was far lower than in the tungsten–nickel–cobalt alloy, indicating an apparent toxic synergy among the three metals. These results, added to those from the study of Kalinich et al. (2005) in which embedded nickel (and nickel-containing) pellets produced malignant tumours in rats, indicate that elemental nickel, whether alone or present in alloyed form with cobalt and tungsten, is carcinogenic.

6. Discussion of pertinent findings

The assertion made by Ogundipe et al. (2007), that tungsten in the environment from discharged shot is toxic, has not been substantiated using the criteria of Strigul et al. (2005) and Dermatas et al. (2004). Even where very heavy gunshot loadings from spent lead shot may be expected and maximum dissolution and bioavailability of tungsten in the shot is assumed, the predicted amounts of tungsten in the soil fall far below the 1% threshold identified as toxic to soil organisms. This conclusion, based on extremely heavy shot loadings by shooters, applies to commercial brands of tungsten-based shot containing 51–95% tungsten by mass.

The manner of soil deposition of metallic tungsten from shot and lead-free bullets made from tungsten may have an important bearing on claims of a toxic tungsten legacy. The studies of Dermatas et al. (2004) and Strigul et al. (2005) were prompted by high tungsten levels in the soils at military rifle training ranges, not areas where gunshot from hunting had fallen. Large numbers of soldiers fire many bullets during training, especially during rapid-fire situations. The bullets are stopped in earthen backstops and, should tungsten remnants accumulate, they could readily exceed the 1% and 3% thresholds identified. Remediation of such training sites, involving the reclamation and recycling of tungsten fragments, is possible because they are both readily-accessible and restricted geographically. By contrast, hunting with shotguns occurs across a far wider geographic area, whether over upland or wetland sites, and so there is a greater dispersion of the non-toxic shot that leads to a far slower rate of metal accumulation at a given location.

The case for soluble tungsten, alone, in potable water causing childhood leukemia (as in the Churchill County situation) has not been substantiated, despite considerable scientific examination of this issue (Rubin et al., 2007). Dosing rats' drinking water with soluble tungsten salts under experimental conditions with amounts of tungsten far above the 'worst-case' aquatic EEC from shot has not led to tumour development.

The experimental testing in ducks of ingested tungsten-based (tungsten–iron and tungsten–polymer) shot under the Tier 3 (150 days exposure across two generations) protocol (US Fish and Wildlife Service (USFWS), 1997), by Mitchell et al. (2001a, 2001b, 2001c), did not report carcinogenicity, despite the solubilization of tungsten and its absorption into the circulation. Tungsten–plastic shot is made from pure tungsten powder mixed with an inert plastic and so relates most closely to the experimental testing of pure tungsten (as opposed to shot types made from tungsten alloys). The results of testing ingested tungsten–plastic shot in ducks are consistent with the results of Barrett et al. (2000), Butler et al. (2000), Peuster et al. (2003a, 2003b) and Bachthaler et al. (2004), in which pure tungsten coils were observed not to cause toxicity in both humans and rabbits. In view of these results, it is suggested that tungsten–plastic shot and other tungsten-based shot taken into the gut of scavenging birds and mammals, and humans who eat shot in the tissues of game, will not cause adverse local or systemic effects.

In North America and Europe, many birds are wounded each year from non-lethal gunfire from waterfowl hunters. The percentage of adult birds carrying shot in the body is given as 29.1% and 20% for two Eider species (*Somateria*) in Greenland (Falk et al., 2006). Hicklin and Barrow (2004) found that 25% of 1624 radiographed waterfowl of different species in Canada contained embedded shot. Tavecchia et al. (2001) reported that up to 29% of Mallard ducks (*Anas platyrhynchos)* captured in a given year in the Camargue of France may contain shot embedded in the muscles. The incidence of embedded shot in adult teal (*Anas crecca*) captured in the same locality was 7.5% for females and 9.6% for males (Guillemain et al., 2007). The prevalence of embedded shot appears to be greater for larger-bodied, and longer-lived, geese than ducks. Pink-footed geese (*Anser brachyrhynchus*) are hunted in Norway and Denmark and, prior to 1997, 25% of juvenile geese and 36% of older birds contained embedded shot in their muscles (Noer et al., 2007). Forty-four percent of 45 trapped Greylag Geese (*Anser anser*) examined by Mateo et al. (2007) in Spain carried embedded shot. Given that so many millions of waterfowl may live for years with lead shot in their body, it is important to determine if the substitutes for lead shot may have a detrimental impact on the birds' existence beyond the initial wounding. From a management perspective, little gain in waterfowl survival is achieved if toxic lead shot is replaced by

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materials that, while non-toxic when ingested, pose risks of carcinogenicity when embedded.

Some of the new brands of tungsten-based shot approved by the US government are allowed to contain up to 40% by mass of nickel alloyed with tungsten (US Fish and Wildlife Service (USFWS), 2006) (Table 1). As suggested by Salnikow and Kasprzak (2005), a high-nickel-content shot, slowly releasing ions from the site of shot implantation over months to years, might create the conditions for genotoxicity and, indeed, the study of Kalinich et al. (2005) has already demonstrated the carcinogenic potency of both metallic nickel and a 6% nickel–tungsten alloy implanted in rat muscle.

It is suggested that protocols for assessing the potential toxicity of lead shot substitutes, such as that of the US Fish and Wildlife Service (USFWS) (1997), be amended to include provisions for the testing of candidate shot by intramuscular implantation to determine if prolonged inflammation or tumour development occurs. Kraabel et al. (1996) investigated the effects of embedding tungsten–bismuth–tin shot into the pectoral muscles of ducks after *8 weeks* (our italics), while Kalinich et al. (2005) showed that the imminent mortality of rats from tumour development attributed to nickel occurred between *weeks 23 and 30* (our italics) post-implantation. Moreover, the duration of regulatory testing for chemical carcinogenicity is typically *18–24 months* (our italics). Notwithstanding the likely differences in response between birds and mammals, 8 weeks may not be long enough to detect possible long-term inflammation and/or carcinogenic effects of metal implantation in birds. Certainly thirty-day testing, as required under Tier 2 conditions of the US Fish and Wildlife Service (USFWS) (1997) protocol, will not allow sufficient time; thus the testing of embedded shot should be made part of Tier 3 conditions and the duration of embedding be made commensurate with the time required to demonstrate non-carcinogenicity of the shot materials.

7. Conclusions

The use of tungsten in lead-free shot is not associated with environmental toxicity, even when such shot are present in soil and water at levels exceeding the heaviest known shot burdens. The tungsten EECs for two brands of commercial shot containing 51% and 96% tungsten fall far below the 1% tungsten threshold that is associated with impacts on soil biota. Extensive medical investigation has not been able to implicate tungsten in potable water as the cause of human leukemia in Nevada, USA. Tungsten is often alloyed with nickel and cobalt, especially for use in military penetrators. Several independent chronic exposure studies have shown that elemental tungsten, whether ingested or implanted in muscle, does not produce tumours or any other pathological condition; however, the nickel present in such alloys is demonstrably carcinogenic when implanted into muscle. Many millions of waterfowl in Europe and North America carry embedded shot in their body as a consequence of non-fatal shooting. The US Fish and Wildlife Service recently approved several types of shot that may contain up to 40% nickel. It is appropriate for regulatory agencies to consider the inclusion of an embedded shot (intramuscular implantation) treatment as a new component of the legal requirements when evaluating new 'non-toxic' shot candidates.

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