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Tissue–Bismuth Levels of Game Birds Harvested with Bismuth Shotshell: Policy Implications

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Bismuth (Bi) is a rare element found in the earth's crust. Commercial Bi is mainly found as a by-product of lead (Pb) and copper (Cu) refining and remains with these metals after being smelted. Physically, Bi metal exhibits a reddish tinge and is a soft, brittle element with a silver-white crystalline structure. It is found in the periodic table in the same family group (VA) as arsenic (As) and antimony (Sb) (Fowler and Vouk 1979; CBP 1999). Chemically, Bi forms compounds in two valencies, 3+ and 5+; of the two, the trivalent cation is the most abundant and stable form (CBP 1999). Although shotshells containing Bi pellets have been approved as a "non-toxic" alternative to Pb pellets for the hunting of game birds in North America, it is not logical to replace one toxic metal (Pb) with another toxic metal (Bi) unless it can be shown conclusively that Bi is benign not only for wildlife but also humans (as described in the CWS guidelines for non-toxic testing; CWS 1993) and the general environment. This is especially true when one considers that neurotoxicity related to Bi compounds has been of interest for over thirty years in humans, ever since the epidemic of encephalopathy in France with over 942 cases and 72 deaths (Slikkerveer and de Wolff 1989) and the 29 cases of Bi-related neurotoxicity reported in Australia in 1973 (Sharma et al. 1994). It is of current interest because of the increase in Bi-containing medications used to fight a variety of gastrointestinal ailments even though the exact etiology of Bi encephalopathy is still unknown. The early signs of Bi-related neurotoxicity include increased emotionality, insomnia, ingestional changes, encephalopathy, motor dysfunction, ataxia, tremors and convulsions (Ross et al. 1994). Bismuth encephalopathy is reported most commonly in patients undergoing long-term, high-dose oral Bi treatments for gastrointestinal problems (Wilson 1994). Bismuth is said to exhibit a wide tissue distribution, which can result in the long-term accumulation of the metal in cases of chronic therapy (Friedland et. al. 1993). Based on animal studies, Bi appears to accumulate in the brain according to Ross et al. (1994). Sharma et al. (1994) state that Bi crosses the blood-brain barrier with the potential to disturb the oxidative cerebral metabolism. Though there is potential for Bi intoxication to become fatal, if left unrecognized (Slikkerveer and de Wolff 1989), a proper diagnosis would lead to termination of Bi therapy and recovery over a period of months concomitant with a decrease in the body burden of this metal (Friedland et al. 1993; Ross et al. 1994). It is surprising that neurotoxicity is widely recognized in the health science literature as a major concern associated with the use of Bi-containing medication, but neurotoxicity testing in game birds was never included in the guidelines established for the testing of non-toxic shotshell substitutes, such as Bi, by the CWS and USFWS (CWS 1993).

Three Bi formulations have been studied recently for non-toxicity in game birds: 100% Bi; Bi-tin (Sn) ("Bi shot"); and the tungsten-Bi-tin shot (TBT). Sanderson et

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al. (1992) placed 2, 4, or 8 no. 2 Bi, Pb, or Fe shot in the proventriculus of game-farm mallards (*Anas platyrhynchos domesticus*). There was "almost no Bi in the blood, none in the muscle, and minute amounts in liver and bones" after a period of 30 days (Sanderson et al. 1992). Comparable results were obtained by Ringelman et al. (1993) for game-farm mallards dosed with 12-17 TBT shot introduced into the esophagus. Comparison of dosed birds with controls found no significant differences in behavior, food consumption, body mass, or Bi concentration in kidney and liver, and there were no histopathological lesions or evidence of toxicity during 32 days of monitoring (Ringelman et al. 1993). In 1997, two major studies were set up to explore the toxicity of the Bi shot. Following the guidelines set by the CWS and USFWS, both acute and chronic toxicity studies were done. The primary objective of the first study was to test the acute (30 days) toxicity of Bi shot placed in the proventriculus of game-farm mallards (Sanderson et al. 1997a). Post-mortem examination of the kidneys and liver showed only slight tissue changes. Measurements on the survival, body weight, hematocrit and weight of the organs were conducted with no toxic affects reported for the Bi exposed group. Thus, Sanderson et al. (1997a) concluded Bi shot did not adversely affect game-farm mallards. The second study had two main objectives: determination of the chronic (150 days) toxicity of Bi shot and determination of the effects of ingested Bi on the reproductive ability of dosed game-farm mallards (Sanderson et al. 1997b). No changes were seen in survival rates or tissue health. No reproductive effects were shown in egg fertility, egg weight, eggshell thickness, or duckling weight. However, hatchability of eggs was reported to be low (Sanderson et al. 1997b). Lastly, two studies of experimentally-embedded shot have concluded that Bi shot is benign, producing only a mild inflammatory tissue response in pectoral muscles (Kraabel et al. 1996; Sanderson et al. 1998). By contrast, a more recent study using silver lactate autometallography has clearly shown that experimentally embedded Bi pellets in tissue of mice are not benign; Bi was seen to enter the nervous system, kidney, liver spleen and lung (Pamphlett et al. 2000). They conclude "it seems prudent to look for pellet material that is more inert as regards the tissue uptake of its components."

In addition to the question regarding the toxicity of Bi, the exact mechanism of Bi absorption in humans has not been characterized (Slikkerveer et al. 1995; Phillips et al. 2000). Krari et al. (1995) states the main hypothesis of Bi absorption is that intestinal flora are involved, although some substances in food or drink enhance Bi absorption. Slikkerveer et al. (1992) add that absorption of Bi is increased by sulfhydryl-group containing compounds like cysteine. Another theory suggests that Bi-containing colloidal particles penetrate through damaged mucosa of the gastric antrum with particle size being of importance (Nwokolo et al. 1992). Hespe et al. (1993) have proposed an alternative theory of Bi absorption based on the physio-chemical behaviour of colloidal metal citrates. If the exact mechanism of Bi absorption is not known, it does not seem prudent to allow Bi shotshell to be used to harvest game birds; people (including children) may be ingesting wild meats contaminated with Bi pellets and fragments, similar to the scenario that has been well documented for lead shotshell (e.g., Tsuji and Nieboer 1997; Scheuhammer et al. 1998). In this paper, we examine whether Bi pellets fragment upon impacting game birds harvested with Bi shotshell and pose a potential unquantified source of Bi for people ingesting wild game.

MATERIALS AND METHODS

During the 1998 spring hunting season, First Nation Cree of the western James Bay region shot two species of upland game birds (*Tympanuchus phasianellus*, sharp-tailed grouse, n=19; *Lagopus lagopus*, ptarmigan, n=1) with Bi-Sn shotshell (12

gauge, no. 4) as part of their normal harvest. They allowed the salvaging of certain parts of the birds (heart, leg, breast, gizzard, liver) for the present study. Upland game birds were chosen as the study species because these birds are not typically wounded with shotshells in the western James Bay region; they are easily harvested with shotshell and even snared on the ground or in the trees (B. Katapatuk, pers. com.). Thus, birds of these species are less likely to have Pb shot previously embedded in their tissue, which would confound our results because Bi is often found in association with Pb (Fowler and Vouk 1979; Jayasinghe et al. unpublished data). Stainless steel blades were used to excise striated muscle from the legs and breasts of the birds; hearts, livers and gizzards (minus gizzard contents and grinding pads) were taken whole. Samples were subsequently sealed in individually marked plastic zip-lock bags in preparation for radiography. Samples were placed on standard radiographic film (43 cm x 35.5 cm) with lead identification tags. Tissue and film were then radiographed (70 kV, 100 mA, 1/20 sec) using a Picker International Inc. radiographic unit (model no. 754-971D) with the film being subsequently processed. Tissue samples with corresponding radiopacities (white objects) in the radiograph were identified. Radiographs with dot-size radiopacities were not included in this group because dot-size radiopacities could not be discerned from artifact. An artifact is normally not present in a radiograph but can be produced by defects in the film, incidental to film processing, etc. After radiography, the zip-lock bags with tissue samples were then stored frozen until preparation for analysis.

Tissue samples were oven-dried to constant weight at 70°C prior to being ground in a spice mill with stainless steel blades. Dry weight (dw) values were used because wet weight (ww) values can vary due to air-drying. Dry weight values were later converted to ww values following Tsuji et al. (1999) to allow for comparison with food consumption guidelines, if available. The following empirical relationships were employed: liver dry weight = 0.374 (ww liver) - 0.95 and striated muscle dry weight = 0.262 (ww striated muscle) - 0.41.

Tissue samples (0.10 g) were digested in 1 mL trace-metal grade HNO_3 (JT Baker, Ultrex) in 1.5-mL microtubes (Sarstedt). Microtubes were sealed with lid locks (DiaMed) and left overnight in a fumehood to allow for initial acid digestion. In the morning, the microtubes were placed in heating blocks (20-holes) and placed in a block heater (Multi-Blok, Lab-Line). Initially, samples were digested at 60°C for one hr, followed by another hr at 80°C. Samples were cooled for approximately 10 mins at room temperature before being centrifuged at 11000 rpm for 10 mins in order to facilitate quantitative transfer. The entire contents of each microtube, including any precipitate, was then transferred into 15-mL test tubes (Pyrex) using distilled double-deionized water (DDW). These samples were diluted to a volume of 4-mL with DDW. Test tubes were then vortexed, followed by placement into cylindrical heating blocks (12-holes) on hot plates. The tissue samples were then digested at 120°C for another 5-6 hrs, until dryness. When dry, the residue was taken up in 5-mL of 0.1% HNO_3. Samples were then vortexed and placed in a test tube rack until the determination of Bi by electrothermal atomic absorption spectrometry (EAAS)(Slikkerveer et al. 1993). Samples were analyzed using a Varian Electrothermal Atomic Absorption Spectrometer (model Spectra AA 220) with graphite furnace, automated sampling apparatus, ultrapure Argon gas, Spectra AA 220 version 2.10 data recording software and graphite tubes (coated; Varian). The spectrometer was set to a wavelength of 223.1 nm, lamp current of 10.0 mA, background correction on, sampling mode set to pre-mix and with a slit width of 0.2 nm. The measurement mode was set to record absorbance peak height. Using the spectrometer software, a temperature program was set for bismuth: (1) 5s ramp to 85°C; (2) 40 s ramp to 95 °C; (3) 10 s ramp to 120 °C; (4) 5 s ramp to 400 °C (5) hold

at 400 °C for 8 s; (6) 0.8s ramp to 2000 °C atomization temperature and holding for 2.0 s; and (7) a cleaning step at 2000 °C for 2 s. The default argon flow was 3.0 L/min and was adjusted during steps 5 and 6 to 0 L/min. The absorbance was only recorded during step 6.

Working standards of Bi (0, 2.5, 5, 10, 20, 60 μg/L) were made from a Bi intermediate standard. The standards were made through the addition of 0 - 1500 μL of the intermediate standard plus 25 μL of a Triton solution (10% in DDW) followed by the dilution to 50 mL with 0.1% HNO_3. The intermediate standard (2000 μg/L) was prepared as follows: dilution of 1 mL of Bi Reference Standard (1000 μg/mL; Ricca Chemical Company) after the addition of 0.72 mL of concentrated trace-metal grade HNO_3 (JT Baker, Ultrex) and dilution to 500mL with DDW.

Approximately 1-mL of the sample digests were decanted into individual sample cups; cups were then placed in the spectrometer sample carousel. Prior to the analysis of tissue samples and during each run, calibration curves were recorded after every ten samples to ensure optimization of the spectrometer. The spectrometer was deemed to be operating within acceptable limits after a calibration when the absorbance for a 10 μg/L Bi standard did not deviate from the nominal value by more than ± 10%. If inconsistencies were apparent between the numbers, the machine was re-checked, re-aligned, and a standard curve run again. Samples and calibration standards were analyzed using a matrix modifier (1000 μg/mL Ni nitrate, VWR Scientific). Tissue sample solutions were run in triplicate and the mean values are reported. With each run, a blank was included. All blanks (n=13) were below the detection limit. No duplicates were run because of the heterogeneity of the tissue samples, with respect to fragmented pellets (Figure 1), as has also been clearly shown by Scheuhammer et al. (1998) for Pb pellets.

Based on the observed inter-experimental standard deviation (σ) for the most dilute calibrating solution, the average detection limit (DL) for liver and muscle tissue is estimated as 0.02 μg/g ww (1σ), 0.04 μg/g ww (2σ) and 0.06 μg/g ww (3σ) corresponding to confidence levels of 84, 98 and 99.9%.

RESULTS AND DISCUSSION

Of the 88 tissue samples radiographed, 28 samples had definitive radiographic evidence of relatively large radiopacities. These samples had Bi levels in the range 0.47 - 2.86 μg/g ww. The 60 samples without definitive radiographic evidence of radiopacities (other than small white dots) had Bi levels in the range 0.01 - 2.79 μg/g ww. This suggests that the large radiopacities were Bi-Sn pellets/fragments and that some small white dots in other radiographs were not artifacts but fine fragments of Bi-Sn pellets. This is supported by the observation that liver and muscle Bi concentrations in birds bagged with lead shotshell were considerably lower than those in the present study (see footnote a of Table 1). Further, 17 of 20 birds (85%) harvested with Bi shotshell had at least one tissue sample with a Bi level > 1.00 μg/g ww and of the 88 tissues sampled, 15 had Bi tissue levels > 1.00 μg/g but ≤ 2.00 μg/g ww, while 22 had Bi tissue levels >2.00 μg/g ww. It is not surprising that Bi-Sn pellets fragment upon impacting wild game as pure Bi is brittle (Fowler and Vouk 1979; CBP 1999). Although it is clear from these data (and Table 1) that the use of Bi-Sn shotshell to harvest game birds contaminates the wild meat, the importance of this finding cannot be put into context because consumption guidelines with respect to Bi-contaminated meats are not available..

Figure 1. Radiograph of a striated muscle sample collected from a sharp-tailed grouse harvested with Bi-Sn shotshell in the western James Bay region of northern Ontario, Canada. Pellet fragments embedded in the tissue are radiopaque (white in the radiograph). The contaminated meat constitutes a potential dietary source of Bi.

Table 1. Bi-tissue levels (μg/g, ww) of game birds harvested with Bi-Sn shotshell.

Species Tissue	$\bar{x}$±sd	range	n
sharp-tailed grouse			
heart	1.24±0.98	0.03-2.86	18
leg	0.72±0.84	0.03-2.27	18
breast	1.45±0.81	0.12-2.61	19
gizzard	1.02±1.00	0.01-2.27	10
liver[a]	0.40±0.49	0.02-1.64	18
ptarmigan			
heart	1.08	-	1
leg	0.05	-	1
breast	0.86	-	1
gizzard	2.06	-	1
liver	0.94	-	1

[a]By comparison, in ducks and geese shot with lead shotshell, liver mean Bi concentrations were between the 1σ and 2σ DL values, with the upper end of the range falling between the 2σ and 3σ DLs (Jayasinghe et al. unpublished data).

It should be emphasized that the contamination of wild meat with Bi-Sn pellets and fragments is of concern because it is an unregulated source of elemental Bi in the diet. Even when Bi-containing medication has been prescribed in regulated doses, negative effects other than neurotoxicity, such as nephropathy, have been documented especially in young children (e.g., Urizar and Vernier 1966). Although intra-muscular injection of Bi-containing medication has been of particular concern for children, ingestion of Bi-containing tablets has also resulted in nephropathy (Urizar and Vernier 1966; Islek et al. 2001). Nevertheless, some researchers have claimed that Bi appears to have few side effects, with toxic effects being reported only for patients taking high doses for extended periods (Wagstaff et al. 1988). It is well documented radiographically for people who subsist on wild game that Pb pellets are commonly located intraluminally and/or in the appendix of the gastrointestinal tract (Reddy 1985; Tsuji and Nieboer 1997). Since Bi-Sn pellets are heavy similar to Pb pellets, they are anticipated to sink in the semiliquid matrix in the cecum and gravitate towards the appendix (Tsuji and Nieboer 1997). Once in this area, the Bi-Sn pellets can accumulate and remain for years. Indeed, over 200 Pb pellets have been found in a person's appendix and one patient was followed for 13 years (Reddy 1985). Therefore, it is reasonable to suggest that Bi-Sn pellets have the potential to accumulate in the appendix and become a chronic source of Bi. In addition, uptake of the Bi^{3+} ion depends strongly on dietary components. For example, citrate in the diet enhances uptake of Bi (Slikkerveer et al. 1993).

Since the exact mechanisms of Bi absorption and neurotoxicity are not well understood, invoking the precautionary principle would appear warranted. Moreover, a non-toxic shotshell replacement for Pb does exist in the form of steel shot. The main element in steel shotshell is an essential element, Fe. Further, steel pellets do not shatter upon impacting wild game and are relatively light, so if steel pellets are accidentally ingested they should be easily voided. Although there has been concern that steel shotshell is ballistically inferior to Pb shotshell (while Bi-Sn is similar to Pb), studies have shown that steel shot can perform comparably to Pb shot if adjustments are made in shooting technique and choice of shotshell size (Morehouse 1992). Thus, it is our opinion that it is best to err on the side of caution.

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