Lead shot contribution to blood lead of First Nations people: 
The use of lead isotopes to identify the source of exposure

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ARTICLE INFO

Article history: 
Received 4 February 2008 
Received in revised form 20 June 2008 
Accepted 25 June 2008 
Available online 3 August 2008

Keywords: 
Lead shotshell 
Subsistence hunting 
Waterfowl 
Lead isotope ratios 
Blood lead 
First Nations

ABSTRACT

Although lead isotope ratios have been used to identify lead ammunition (lead shotshell pellets and bullets) as a source of exposure for First Nations people of Canada, the actual source of lead exposure needs to be further clarified. Whole blood samples for First Nations people of Ontario, Canada, were collected from participants prior to the traditional spring harvest of water birds, as well as post-harvest. Blood-lead levels and stable lead isotope ratios prior to, and after the harvest were determined by ICP-MS. Data were analyzed by paired t-tests and Wilcoxon Signed-Ranks tests. All participants consumed water birds harvested with lead shotshell during the period of study. For the group excluding six males who were potentially exposed to other sources of lead (as revealed through a questionnaire), paired t-tests and Wilcoxon Signed-Ranks tests showed consistent results: significant (p<0.05) increases in blood-lead concentrations and blood levels of 206Pb/204Pb and 206Pb/207Pb towards the mean values we previously reported for lead shotshell pellets; and a significant decrease in 208Pb/206Pb values towards the mean for lead shotshell pellets. However, when we categorized the group further into a group that did not use firearms and did not eat any other traditional foods harvested with lead ammunition other than waterfowl, our predictions for 206Pb/204Pb, 206Pb/207Pb and 208Pb/206Pb hold true, but there was not a significant increase in blood-lead level after the hunt. It appears that the activity of hunting (i.e., use of a shotgun) was also an important route of lead exposure. The banning of lead shotshell for all game hunting would eliminate a source of environmental lead for all people who use firearms and/or eat wild game.

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1. Introduction

Lead is a toxic metal that exerts its effects on bodily systems even at low levels of exposure (ATSDR, 2005; CDC, 2005). The use of lead shotshell to harvest game birds has been suggested as a source of lead in humans (Johansen et al., 2006; Levesque et al., 2003; Tsuji and Nieboer, 1997) through the consumption of lead pellets and/or fragments that contaminate the meat (Scheuhammer et al., 1998; Tsuji et al., 1999). However, a recent blood-lead study of First Nations people in Canada revealed using lead isotope ratios that although the source of lead exposure for the First Nations people could be identified as lead ammunition, the isotope ratios for lead shotshell pellets and bullets were indistinguishable (Tsuji et al., 2008a). It was concluded that lead-contaminated meat from

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doi:10.1016/j.scitotenv.2008.06.048
game harvested with lead bullets may also be contributing to the lead body burden (Tsuij et al., 2008a).

Owing to the seasonal nature of harvesting activities for aboriginal groups (Bjerregard et al., 2004; Johansen et al., 2006; Kosatsky, 1998; Tsuij and Nieboer, 1999), blood-lead levels should vary with the harvesting seasons. Blood-lead levels should be lowest prior to the bird-harvesting season and highest afterwards, if game birds harvested with lead shotshell are a major source of lead for subsistence harvesting groups (Bjerregard et al., 2004; Johansen et al., 2006; Kosatsky, 1998). In a study by Kosatsky (1998), it was shown that blood-lead concentrations increased two months following the goose hunting season in eastern James Bay Cree of northern Quebec, Canada. Similarly, in native Greenlanders (Inuit with some European genes; Bjerregard et al., 2004), Johansen et al. (2006) have reported a seasonal variation in blood-lead levels, which were highest when bird consumption peaked; however, some participants had relatively high blood-lead concentrations but low bird intakes. Johansen et al. (2006) suggested the use of stable lead isotope ratios as a way to distinguish between sources of lead.

Lead has four stable isotopes: three that are radiogenic (206Pb is from the radioactive decay of 238U; 207Pb from 235U; and 208Pb from 232Th), and one non-radiogenic isotope (204Pb; Rabinowitz, 1995). In the present study, we first predict that lead-contaminated food must be substantial to be seen as a change in blood-lead concentration and/or isotope ratio. Graziano et al. (1996) and Maddaloni et al. (1998) to differentiate between sources of environmental lead when sample size has been limited. In this paper, we examine blood-lead levels and stable lead isotope ratios prior to, and after the traditional spring harvest of water birds by First Nation Cree of the western James Bay region, northern Ontario, Canada, to determine whether such birds harvested with lead shotshell constitute a major source of lead for this subsistence harvesting group. The consumption of lead-contaminated food must be substantial to be seen as a change in blood-lead concentration and/or isotope ratio (Gulson et al., 1999). In the present study, we first predict that there should be an increase in blood-lead concentrations post-spring harvest. Second, since blood is a mixture of both contemporaneous sources of environmental lead and endogenous bone contribution (Gulson et al., 1995; Smith et al., 1996), we predict that blood-lead isotope ratios after the spring harvest should vary in the direction of the isotope ratios of lead shotshell pellets when compared to pre-spring harvest values. Indeed, 24-hour, human (n = 6) dosing experiments by Graziano et al. (1996) and Maddaloni et al. (1998) have shown that ingested lead can impact pre-dosing blood-lead isotope ratios such that post-dosing, blood-lead isotope ratios varied towards the lead isotope ratio of the dosing agent.

2. Methods

2.1 Study sites

First Nation Cree that participated in the present study resided in the remote western James Bay area of northern Ontario, Canada. Sources of environmental lead were limited in this region. Both water and soil-lead concentrations have been reported as being low, with air levels below the Ontario Ambient Air Quality Criterion of 5 µg/m3 (OMHE, 1989). Most dwellings were built after the lead content in paint had been reduced (Tsuij et al., 1999). Until the 1990s, most First Nations houses lacked indoor plumbing, and when indoor plumbing was installed, copper and plastic piping and fittings were used, as well as lead-free solder (Tsuij et al., 2000). Surface water in the region has been reported to contain a minimal amount of lead (McCrea and Fischer, 1986). Lastly, the use of white lead (89% PbCO₃, 11% linseed oil) for major repairs of wood and canvas boats was not of concern, as fibreglass boats have grown in popularity; while, people were aware of the toxicity of this type of lead and had taken proper precaution with respect to storage and use of this product (Fortin and Decou, 1995).

2.2 Sample collection

This study was approved by the McMaster University Research Ethics Board. All participants signed a consent form prior to the initial blood collection and then completed a questionnaire in an interview format (to collect personal data pertaining to demographics, lifestyle, and the level of consumption of traditional foods over the specific period of the study), after the second blood sample was collected. The hunting of waterfowl during the spring-harvest season (typically mid-April to the end of May) is a way of life for the Cree. Whole blood samples were collected from participants (females, n = 10; males, n = 14) prior to the spring harvest of 2000 (i.e., late March to early April) in lavender-top (with EDTA), 6-ml plastic Vacutainer tubes. Blood samples were mixed, allowed to cool to room temperature, then frozen, and stored at −20 °C. Also, whole-blood samples were collected from participants after the spring harvest and the threat of community flooding had subsided (i.e., early July of 2000; approximately one-and-a-half months after the end of the spring-waterfowl harvest). Post-harvest blood samples were collected from 6 of the original 10 females sampled and 11 of the original 14 males; these participants constitute our cohort. People who were not resampled were absent during the re-sampling period. One female participant who donated pre- and post-harvest blood samples was excluded from the study, because her blood-lead concentration was < 10 µg/L (our detection limit based on three times the average standard deviation of noise); thus, isotope values could not be determined using our protocol. Blood samples were shipped frozen to the Centre de toxicologie, Institut national de sante publique du Quebec for lead and isotope determinations.

2.3 Blood-lead determination

Blood-lead determination was by electrothermal atomic absorption spectrometry (Perkin Elmer model ZL 4100). Samples were simply diluted and injected into the instrument (see Tsuij et al., 2008b for a detailed account). Accuracy and precision were checked using reference material from the Centre de toxicologie’s administered interlaboratory comparison program; while, periodic evaluations were also performed.
using external proficiency testing (e.g., US Centers for Disease Control and Prevention).

2.4. Stable lead isotope determination

Lead isotope ratios were determined by inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer Sciex Elan 6000) and relative abundances were expressed as the ratios $^{206}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$. Operating conditions and mass spectrometer settings were as follows: RF power=1000 W; nebuliser gas flow rate=1.0 L/min; auxiliary gas flow rate=1.0 L/min; plasma gas flow rate=15 L/min; plasma mass spectrometry (ICP-MS; Perkin Elmer Sciex Elan 6000) and relative abundances were expressed as the Pb isotope ratios $^{206}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$. Operating conditions and mass spectrometer settings were as follows: RF power=1000 W; nebuliser gas flow rate=1.0 L/min; auxiliary gas flow rate=1.0 L/min; plasma gas flow rate=15 L/min; nebuliser type was cross flow; interface cones were platinum; dwell time=25 ms; number of sweeps=500; and number of replicates=4 (Tsuji et al., 2008a). Analytical accuracy was assessed using the Pb standard NIST SRM-982 ($n=22$; %RSD: $206/204=0.27; 206/207=0.25; 206/206=0.13$); while, the blood material reference Quebec Multielement External Quality Assessment Scheme 99BO1 was employed.

Simply, blood-lead samples ≥80 μg/L were diluted 10 fold in 0.1% Triton-X100 and 0.5% ammonia solution. To avoid nebulisation problems, a minimum 10-fold dilution was required. For blood-lead samples <80 μg/L, treatments were needed to eliminate some parts of the matrix; acidic deproteination removed most of the organic material in order to eliminate clogging formation during the nebulising process.

2.5. Statistical analyses

The questionnaire revealed that some of the participants had been involved in hobbies/work (after the first blood collection, but prior to the second blood collection) known to be associated with lead exposure (e.g., boat repairs [the use of basic lead carbonate in oil; Fortin and Decou, 1995] and renovation of old buildings where leaded paint was used); these individuals (all males, $n=6$) were analyzed separately as a group, as lead exposure was potentially not just from the consumption of lead-contaminated meat. For this group of males, paired $t$-tests (two-tailed, as we make no predictions) were performed for blood-lead concentration and isotope ratios $^{206}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$; also, data were analyzed by Wilcoxon Signed-Ranks tests (two-tailed). Data analyses were similar for the main group, except, tests were one-tailed. Power achieved for the effect size observed in paired $t$-tests was calculated according to Faul et al. (2007).

3. Results

All participants consumed waterfowl harvested with lead shotshell (range: 8–72 portions; each portion=75 g) during the

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>Sub-group</th>
<th>Subject ID</th>
<th>Sex</th>
<th>Blood Pb (μg/L) Before</th>
<th>Pb isotope 206/204 Before</th>
<th>Pb isotope 206/207 Before</th>
<th>Pb isotope 208/206 Before</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: lead shotshell exposure</td>
<td>a) non-hunter</td>
<td>1008</td>
<td>Female</td>
<td>33</td>
<td>18.38</td>
<td>1.182</td>
<td>2.061</td>
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<td></td>
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<td>1.179</td>
<td>2.065</td>
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<tr>
<td></td>
<td></td>
<td>1529</td>
<td>Male</td>
<td>33</td>
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<td>1.167</td>
<td>2.078</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6000</td>
<td>Male</td>
<td>73</td>
<td>18.52</td>
<td>1.183</td>
<td>2.054</td>
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<tr>
<td></td>
<td></td>
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<td>1.200</td>
<td>2.046</td>
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<td></td>
<td></td>
<td>1043</td>
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<td>1.182</td>
<td>2.061</td>
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<tr>
<td></td>
<td></td>
<td>1047</td>
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<td>41</td>
<td>18.93</td>
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<td>2.020</td>
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<td></td>
<td></td>
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<td>18.73</td>
<td>1.201</td>
<td>2.041</td>
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<tr>
<td></td>
<td></td>
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<td>41</td>
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<td>1.187</td>
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<tr>
<td></td>
<td></td>
<td>1550</td>
<td>Male</td>
<td>41</td>
<td>18.56</td>
<td>1.193</td>
<td>2.045</td>
</tr>
<tr>
<td>Group 2: lead shotshell exposure</td>
<td>b) hunter</td>
<td>1501</td>
<td>Male</td>
<td>178</td>
<td>18.56</td>
<td>1.190</td>
<td>2.050</td>
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<td></td>
<td></td>
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<td>17.95</td>
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<tr>
<td></td>
<td></td>
<td>6001</td>
<td>Male</td>
<td>35</td>
<td>18.18</td>
<td>1.175</td>
<td>2.067</td>
</tr>
</tbody>
</table>

* Samples were collected in late March and early April of 2000, prior to the start of the spring harvest of waterfowl. The spring harvest was from mid-April to the end of May.

b) Samples were collected in early July of 2000, approximately one-and-a-half months after the spring harvest of waterfowl was finished.

c) These participants consumed waterfowl harvested with lead shotshell (April–June, 2000), but did not consume any other game harvested with lead ammunition and did not participate in any other activity during the study period that would have exposed them to other sources of lead, including hunting.

d) These participants consumed waterfowl harvested with lead shotshell and consumed other game harvested with lead ammunition, while participating in the activity of hunting. However, this group did not participate in other activities which would have exposed them to additional sources of lead.

e) These participants consumed waterfowl harvested with lead shotshell and consumed other game harvested with lead ammunition, while participating in the activity of hunting and other activities which may have exposed them to additional sources of lead during the study period (e.g., boat repairs using basic lead carbonate in oil, renovation of old buildings where lead paint was used).
months of April, May, and June of 2000, except for subjects numbered 1008 and 1043 (they consumed waterfowl only in April and May). Large mammals were also consumed (8/16 people; range: 1–48 portions), as well as fish (6/16 participants; range: 1–36). Small mammals (2/16 individuals; range 1–2 portions) and upland game birds (only one person, 3 portions) were rarely eaten during this time period. Twelve of the 16 people in the study participated in the spring harvest; the four individuals (ID # 1008, 1014, 1529, 6000; Table 1) who did not participate in the spring hunt, also did not eat upland game birds, fish, large mammals and only one ate a small mammal (one portion; method of harvest was a snare), but did consume waterfowl harvested with lead shotshell.

Blood-lead concentrations and isotope ratios in participants (including the six males potentially exposed to other lead sources) before and after the spring harvest of waterfowl are given in Table 1. Paired t-tests (Table 2) and Wilcoxon Signed-Ranks tests (Table 2) showed consistent results: significant increases in blood-lead concentration, $^{206}\text{Pb}/^{204}\text{Pb}$, and $^{206}\text{Pb}/^{207}\text{Pb}$ in participants from the first to the second sample, which were accompanied by a significant decrease in $^{208}\text{Pb}/^{206}\text{Pb}$ values. By contrast, paired t-tests and Wilcoxon Signed-Ranks tests both showed no significant increases in blood-lead concentration, $^{206}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ values for the group of six males potentially exposed to other lead sources (Table 2).

### 4. Discussion

Blood-lead concentration results were consistent with the hypothesis that lead-contaminated game bird meat is a significant source of exposure for First Nation Cree of the western James Bay region, a conclusion supported by the significant increase in blood-lead concentrations of our main study group after the spring harvest of water birds, as predicted; while, that of males potentially exposed to other sources of lead did not increase significantly. Similarly, Kosatsky (1998) reported increases in blood-lead levels two months following the spring and fall harvests of waterfowl in eastern James Bay Cree for all age groups (0–5 years, 6–11, 12–19, 20–49, 50+) after test year and sex adjustments. Johansen et al. (2006) have also reported

<table>
<thead>
<tr>
<th>Table 2 – Tests of difference in blood-lead levels and lead isotope ratios in First Nations people before and after the waterfowl hunting season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure group</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Group 1: lead shotshell exposure</td>
</tr>
<tr>
<td>(1-tailed test)</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Group 1a: non-hunter</td>
</tr>
<tr>
<td>(1-tailed test)</td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Group 1b: hunter</td>
</tr>
<tr>
<td>(1-tailed test)</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Group 2: lead shotshell exposure and additional lead exposure</td>
</tr>
<tr>
<td>(2-tailed test)</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

The bolded values are significant (p<0.05).

**a** Samples were collected in late March and early April of 2000 (before the start of the spring harvest of waterfowl) and in early July of 2000, approximately one-and-a-half months after the spring harvest of waterfowl was finished. The spring harvest was from mid-April to the end of May.

**b** Power achieved for the effect size observed in paired t-tests was calculated according to Faul et al. (2007).

**c** These participants consumed waterfowl harvested with lead shotshell (April–June, 2000).

**d** These participants consumed waterfowl harvested with lead shotshell, but did not consume any other game harvested with lead ammunition and did not participate in any other activity during the study period that would have exposed them to other sources of lead, including hunting.

**e** These participants consumed waterfowl harvested with lead shotshell and consumed other game harvested with lead ammunition, while participating in the activity of hunting. However, this group did not participate in other activities which would have exposed them to additional sources of lead.

**f** These participants consumed waterfowl harvested with lead shotshell and consumed other game harvested with lead ammunition, while participating in the activity of hunting and other activities which may have exposed them to additional sources of lead during the study period (e.g., boat repairs using basic lead carbonate in oil, renovation of old buildings where lead paint was used).
a seasonal variation in blood-lead levels where levels were highest when bird consumption was at its highest. The consumption of lead in the diet must be substantial to be seen as a change in blood-lead concentration and/or isotope ratio because of low absorption and the clearance rate of lead from blood (Gulson et al., 1999).

Isotope ratios $^{206}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ showed no significant trends for the special group of six males who were potentially exposed to alternative sources of lead (group 2, Table 2). However, isotope ratios for our main study groups behaved in the way we predicted in that $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{206}\text{Pb}/^{207}\text{Pb}$ values increased significantly after the spring harvest. Values of these isotope ratios varied towards the mean values we have reported for lead shotshell pellets used in the region ($^{206}\text{Pb}/^{204}\text{Pb}=19.19$, $^{206}\text{Pb}/^{207}\text{Pb}=1.223$) and away from the mean levels we have found for atmospheric lead of the region ($^{206}\text{Pb}/^{204}\text{Pb}=18.19$, $^{206}\text{Pb}/^{207}\text{Pb}=1.172$; Tsuji et al., 2008a); while, $^{208}\text{Pb}/^{206}\text{Pb}$ values significantly decreased post-harvest towards the mean level found for lead shotshell pellets ($^{208}\text{Pb}/^{206}\text{Pb}=2.011$) and away from the mean level we have reported for atmospheric lead of the western James Bay region ($^{208}\text{Pb}/^{206}\text{Pb}=2.081$; Tsuji et al., 2008a). Likewise, in a 24-hour dosing experiment by Graziano et al. (1996) of six healthy adults (2 males, 4 females; 23–28 years of age) with lead in the form of sherry stored in lead crystal decanters ($^{208}\text{Pb}/^{206}\text{Pb}$ of the lead crystal decanter = 1.078), $^{208}\text{Pb}/^{206}\text{Pb}$ values decreased significantly from a mean of 1.202 to 1.137. Maddaloni et al. (1998) have also reported for six healthy adults (2 males, 4 females; 23–28 years of age) who were dosed (24-hour experiment) with 250 $\mu$g Pb/70 kg body weight (lead was in a soil matrix delivered in a gelatin capsule; $^{208}\text{Pb}/^{206}\text{Pb}=1.1083\pm0.0002$) that average $^{208}\text{Pb}/^{206}\text{Pb}$ values dropped from 1.195 to 1.167; when the subjects had been fed, the change was in the direction of the $^{206}\text{Pb}/^{207}\text{Pb}$ value of lead in-soil, but the trend was not as dramatic.

Testing of paired (before versus after spring harvest) observations offers maximum statistical power in a dataset with relatively small sample sizes. Nevertheless, observed power of the t-tests for blood lead in Group 1 and Group 1b was below 0.80. This casts some doubt on the significant differences found in comparison of paired individuals before and after the hunt; however, the confirmatory significant result for the Wilcoxon test supports the rejection of the null hypothesis of no difference in blood lead before and after the hunt for these groups of individuals.

Although all our predictions were shown to be correct, the issue of confounders must also be addressed. There are three confounders of interest: 1. Fumes from lead bullets and primers have been recognized as a source of lead exposure for people handling firearms (Anderson et al., 1997; Bonanno et al., 2002; Valway et al., 1989). 2. Hand-to-mouth activities have also been cited as a source of concern (Bonanno et al., 2002; Valway et al., 1989). People who hunted (group 1b) would be exposed to this source of lead; while, the people who did not hunt (group 1a) would not be exposed to the primer. The use of lead shotshell for the hunting of waterfowl was banned in 1991, in the US (USFWS, 1988) and for migratory bird hunting in Canada, in 1999 (Environment Canada, 2000). Nevertheless, lead shotshell can still be used legally for the harvesting of upland game birds and small mammals in North America. In Canada, there are no federal laws restricting the use of lead shotshell in this capacity because upland game falls under the jurisdiction of the provincial governments (see for e.g., Ontario Provincial Offences, 1999); only migratory game birds are regulated by the federal “non-toxic” shot policy (Environment Canada, 2000). A similar situation exists in the US with the separation of power between federal and state governments (Thomas, 1997). Moreover, it has been shown that if lead shotshell is still legally available for upland game hunting, lead shotshell will also be used for waterfowl hunting in remote areas (Balogh, 1999). Clearly, any game harvested with lead shotshell may become contaminated to the point where the meat is unsuitable for human consumption. There is also the issue of additional lead exposure through the use firearms. The banning of lead shotshell for all game hunting would eliminate a significant source of environmental lead, especially for subsistence hunting groups.

Acknowledgements

We thank all the participants and Chief and Councils. We also acknowledge funding from the Toxic Substances Research Initiative (Health Canada).

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