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Preliminary Results of a Survey of Lead Levels in Human Liver Tissue

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Lead and compounds containing lead are widespread throughout the environment. Aside from specific instances of occupational exposure (for example, workers in smelters, radiator repair shops, battery manufacturing plants), there are several routes by which the general population is exposed to lead. Humans are exposed to trace quantities of lead on a daily basis through inhalation of air, consumption of drinking water, and inigestion of food. Children, who tend to have increased hand-to-mouth activity, ingest larger quantities of lead-containing dust and dirt than adults. Although direct contact is made daily with dust, dirt, and painted surfaces containing lead, very little lead is adsorbed through the skin.

Lead generally enters the body via the lungs or gastrointestinal tract and subsequently enters the bloodstream. It has been estimated that young children absorb 42% of the lead that reaches the gastrointestinal tract as compared to the 5 to 15% absorbed by adults (Goyer 1991). Children also tend to retain a much higher proportion of absorbed lead than adults, typically greater than 30% versus less than 5%, respectively. Most of the lead which is stored by the body is found in bone and teeth. Lead which is not stored by the body is eliminated in urine and feces.

Lead has long been recognized as a potential hazard to human health. The effects of lead appear to be the same regardless of the mechanism by which it enters the body. Lead affects almost every organ and system, in particular the central nervous system (Agency for Toxic Substances and Disease Registry 1993). Fetuses, through maternal exposure, and young children are especially susceptible to the harmful effects of lead. Although the scientific and medical communities have long realized that lead is particularly harmful to unborn and young children, there is recent evidence to suggest that measurable and possibly even irreversible damage may occur at much lower levels of exposure. For this reason, the U.S. Center for Disease Control recently lowered its “threshold of concern” blood lead level to 10 µg/dL (Agency for Toxic Substances and Disease Registry 1993).

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Health Canada (1994) similarly recommended a blood lead intervention level of 0.5 µmol/L (10.4 µg/dL).

In recent years, steps have been taken to minimize exposure of the general population, especially children, to lead. Unleaded fuels were introduced in Canada in 1975. Airborne lead concentrations dropped dramatically (76% on average) from 1973 to 1985 as the use of unleaded fuels increased. In 1990, the use of leaded gasoline in motor vehicles was prohibited in Canada (Health Canada 1990). Dietary intake of lead has also been reduced since the use of lead solder in food canning processes and plumbing of drinking water supplies has been restricted. In 1976, the Hazardous Products Act limited the amount of lead permissible in interior paint to 0.5 percent by dry weight (Health Canada 1990).

Data collected from provincial and municipal studies carried out in Ontario suggest that blood lead levels are decreasing in adults and children representative of the general population (Health Canada 1994). Much of this observed decrease in blood lead levels has been attributed to the elimination of lead-based additives in gasoline and lead solder in commercial food canning.

There is little information regarding the impact of chronic exposure to lead over the lifetime of an individual. While animal studies have shown that lead acetate and lead phosphate may be carcinogens, there is no conclusive evidence linking lead to cancer in humans (Agency for Toxic Substances and Disease Registry 1993). Zeisler et al. (1984) performed multielemental analyses, including the determination of lead, on 36 liver tissue samples collected as part of a pilot project on specimen banking. Lead concentrations were found to range from approximately 0.1 to 1.3 µg/g. Keinonen (1992) compared the isotopic composition of lead found in human liver, lung, and bone to that found in various environmental indicators of sources of lead pollution. Emissions from incinerators and lead smelters were suggested to be the major factors influencing the levels of lead found in the human tissue samples which were analyzed. Bona and co-workers (1992) examined the levels of the heavy metals chromium, manganese, nickel, and lead in liver samples obtained from 44 victims of sudden traumatic death. The average hepatic lead concentration was found to be 4.43 ppm (dry weight) with a standard deviation of 3.12 ppm. Concentrations of lead, cadmium, and zinc were determined in various reproductive organs, liver and kidneys from 41 men who had died suddenly (Oldereid et al. 1993). There was no significant correlation between blood lead levels and organ concentrations nor between concentrations and age. Gerhardsson and co-workers (1995) determined the concentration of lead in various tissue samples collected from deceased lead smelter workers. Lead concentrations in liver, lung, kidney, brain, hair, and nail samples collected for 32 long-term smelter workers were compared to those obtained for a group of 10 control males. Of the four organs sampled, the highest lead levels were found in the liver for both groups of deceased men. The organs

of the smelter workers were found to contain higher levels of lead than the corresponding organs of the control group, the largest relative difference being observed for the brain.

In a recent study by Castilla (1995), the relationship between blood and liver lead levels and liver function in patients with liver disease were investigated. As with the earlier study by Oldereid (1993), no statistically significant relationship was determined to be present for lead concentrations in blood and liver samples. While blood lead levels could be linked to alcohol consumption and alcohol-related liver disease, the same relationship was not observed for hepatic lead concentrations. Liver function did not appear to influence either blood or hepatic lead levels.

It was the intention of this study to obtain preliminary data in order to establish a normal range of hepatic lead concentrations from individuals representative of the general population of Saskatchewan.

MATERIALS AND METHODS

Portions of autopsied human liver specimens from deceased residents of the Province of Saskatchewan (Canada) were collectcd and stored in a freezer for future analysis. A total of 73 samples, 42 males and 31 females, were subjected to the sample preparation procedure described herein. Approximately 1 to 2 grams of wet liver tissue were weighed into a 25-mL round-bottomed tube. The liver was gently dried at 60 °C for 90 hours and the resulting dry weight was recorded. Randomly selected samples were processed in duplicate to demonstrate the precision afforded by the employed methodology. The specimens were digested in 5 mL of nitric acid for a period of 72 hours at room temperature. The digested/acidified liver samples were diluted as necessary to permit concentration interpolation. All reagent blanks were prepared and treated in exactly the same manner.

A Varian SpectrAA-300Z atomic absorption spectrophotometer (Victoria, Australia) equipped with a graphite furnace and Zeeman background correction system was used in conjunction with a programmable Varian autosampler. The hollow cathode Pb lamp and the pyrolytically coated partition graphite tubes were purchased from Varian. The analytical resonance wavelength at 283.3 nm and a slit width of 0.5 nm were utilized for all analyses. A current of 5 ma was applied to the hollow cathode Pb lamp. The graphite furnace program used for all lead determinations is shown in Table 1. Extra steps were employed at the termination of the atomization furnace program to allow the graphite tube to cool slowly to 40 °C and thus prevent spattering of the subsequent injection. Along with this modification, an extended dispensing rate (with heated injection, 40 °C) was used to ensure that all the dispensed liquid would be slowly introduced to be contained within the platform cavity for subsequent temperature programming. The

Table 1. GFAAS temperature program for the determination of lead in human autopsied liver samples

STEP NO.	TEMP (^{0}C)	RAMP TIME (sec)	HOLD TIME (sec)	Ar GAS FLOW (L/min)
1	120	65	20	3.0
2	400	15	7	3.0
3	2600	1	7	0
4	40	13	2	3.0

automatic sampler was programmed to deliver 5 µL of solution to the graphite tube.

As part of our routine in-laboratory quality assurance protocol for the determination of lead in blood, certified control blood is analyzed along with the samples. In order to verify the performance of the entire analytical procedure used for the determination of lead in liver tissue, a bovine liver standard reference material (SRM) was analyzed in replicate along with the human liver samples. The SRM 1577a consisted of lyophilized bovine liver and was obtained from the National Institute of Standards and Technology (Gaithersburg, MD). Approximately 0.5 g of the SRM liver were processed with each set of samples. A total of 10 analyses were performed on the SRM. The certified concentration of lead in the desiccated bovine liver is 0.135 ± 0.015 µg/g.

RESULTS AND DISCUSSION

Liver tissue samples from a total of 73 humans (42 males and 31 females) were dried, subjected to acid digestion, and subsequently analyzed for lead using GFAAS. The ages of the subjects ranged from 5 months to 88 years for the males and 2 months to 86 years for the females. The hepatic lead concentrations for the male and female subjects are given in Tables 2 and 3, respectively.

Of the 42 liver samples collected from deceased males, there was one sample which clearly had an elevated lead concentration (number 31 in Table 2). Given that the lead concentration was much higher than in any of the liver samples, the analysis was repeated using another portion of tissue. This second analysis confirmed the initial result to within 7%. If this liver tissue sample is excluded from the data set, the average hepatic lead concentration was determincd to be 0.53 µg/g with a standard deviation of 0.41 µg/g. If the sample (number 24 in Table 2) with next highest hepatic lead concentration (2.5 µg/g) was excluded, the average lead level would become 0.48 ± 0.29 µg/g.

Table 2. Hepatic lead concentrations in male humans

NO.	AGE (years)	Pb LEVEL (µg/g; dry wt.)	NO.	AGE (years)	Pb LEVEL (µg/g; dry wt.)
1	0.42	0.91	22	43	0.59
2	1	0.20	23	44	0.84
3	5	0.13	24	45	2.5
4	16	0.16	25	49	0.38
5	16	0.33	26	50	0.18
6	17	0.22	27	51	0.01
7	19	0.14	28	56	0.60
8	20	0.71	29	57	0.64
9	24	0.61	30	58	0.16
10	26	0.68	31	62	7.2
11	26	0.24	32	65	0.82
12	28	0.59	33	66	1.1
13	29	1.1	34	68	0.41
14	32	0.86	35	71	0.50
15	33	0.10	36	73	0.96
16	33	0.37	37	74	0.30
17	35	0.34	38	75	0.31
18	37	0.63	39	77	0.68
19	39	0.15	40	80	0.79
20	39	0.21	41	85	0.66
21	41	0.31	42	88	0.81

In the case of the liver samples collected from deceased females, a smaller range of hepatic lead concentrations was found, ranging from 0.02 to 1.2 µg/g. Based upon the results obtained for 31 samples, the average concentration of lead found in female human liver tissue was determined to be 0.25 ± 0.24 µg/g.

Based upon the inspection of the data in Tables 2 and 3, there are no apparent trends between age and hepatic lead concentration for either sex. The lack of any

Table 3. Hepatic lead concentrations in female humans

NO.	AGE (years)	Pb LEVEL (µg/g; dry wt.)	NO.	AGE (years)	Pb LEVEL (µg/g; dry wt.)
1	0.17	0.04	17	54	0.31
2	2	0.19	18	56	0.21
3	14	0.09	19	57	0.23
4	27	0.44	20	58	0.38
5	29	1.2	21	62	0.20
6	31	0.28	22	63	0.08
7	33	0.16	23	63	0.40
8	33	0.10	24	66	0.26
9	37	0.12	25	69	0.16
10	39	0.06	26	72	0.09
11	40	0.02	27	76	0.39
12	40	0.11	28	78	0.21
13	41	0.13	29	80	0.28
14	42	0.38	30	81	0.22
15	48	0.82	31	86	0.43
16	51	0.06			

relationship between the age of the deceased individual and the amount of lead found in his/her liver is consistent with the findings of Bona (1992) and Oldereid (1993). Livers from males tend to contain a higher level of lead on average than for females. In fact of the 42 liver samples collected from deceased males, only 1 contained less than 0.1 µg/g of lead. Out of 32 female liver samples, 7 were found to contain less than 0.1 µg/g of lead. A much larger number of samples would have to be analyzed to determine the statistical significance of this apparent difference in hepatic lead levels.

As mentioned above, there were two individuals having hepatic lead concentrations which were considerably higher than the others. The liver sample of a 62-year old male was found to contain 7.2 µg/g of lead, while that of a younger man (45 years) had 2.5 µg/g. Investigation into the former individual's case history indicated that the person had resided in the northern part of the province of Saskatchewan and was known to have hunted wild game for the

purpose of consumption. Physicians in a remote northern Ontario community reported observing lead shot in the gastrointestinal tract of adults who had inadvertently consumed whole shot with wild meat (Smith and Rea 1995). It is speculated that dietary lead may have contributed significantly to the elevated hepatic lead level found for this individual. The second individual, having a hepatic lead concentration of 2.5 µg/g, was an electrician. It is quite likely that this individual would have employed lead-containing solder on a routine basis. He therefore would have been exposed to lead fumes and dust during handling and use of the solder.

The use of a standard reference material for which a certified analyte concentration has been established provides an excellent means of verifying the accuracy of the analytical procedure which has been employed. In the analysis of 10 replicate samples of the bovine liver SRM, the concentration of lead was found to range from 0.121 to 0.149 µg/g. The mean lead concentration (based on 10 replicates) was determined to be 0.136 ± 0.009 µg/g. The analytical procedure provided results which were both accurate and precise since all 10 analyses of the SRM liver yielded results which were within the established range of acceptable lead concentrations (i.e., 0.135 ± 0.015 µg/g).

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