Graphite Furnace Atomic Absorption Spectrometry: Lead Contamination in Vermont Maple Syrup

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Abstract

As humans have increasingly utilized lead (Pb) and lead-based compounds, lead poisoning has become a persistent threat to human health. To minimize human exposure to lead in recent decades, measures have been taken to remove it from paint and gasoline. However, lead still remains a low-level toxin as a result of other sources. One of the principle sources of lead uptake in the human body today is food contaminated by processing and canning methods. Exposure due to ingestion can cause low-level lead toxicity. Developmental deficits in children, attention disorders, and high blood pressure have all been linked to this low-level lead exposure.

Recently, investigations have focused on maple syrup as one of the sources of ingested lead. The purpose of this project was to use the Graphite Furnace Atomic Absorption Spectrometer to evaluate samples of maple syrup from four different maple syrup production sites in Vermont and to determine whether or not these syrup samples contain considerable amounts of lead. In the four maple syrup samples tested, the lead concentrations were determined to be between 58.00 and 111.73 ppb. Because these are lower than the acceptable standard level for lead in maple syrup set at 250 ppb, these concentrations are not large enough to pose a health risk.

Biological Effects of Lead Exposure on Humans

The effects of lead exposure on the human body are diverse and abundant. Lead can affect blood pressure, cause cardiovascular disease, impair growth and development in children, and alter the visual and auditory systems of both children and adults. Lead correlates with a decrease in a child’s IQ, and it can also have neurobehavioral, neuropathological, neurophysiological, and neurochemical manifestations at a variety of dosages (Silbergeld 1992). Many exposed humans who do not overtly display high-level acute lead poisoning symptoms, such as vomiting, drowsiness, imbalance, convulsions, or coma still show long-term deficits that can be attributed to lead exposure (Lin-Fu 1985).

At the cellular level, three mechanisms for lead toxicity have been hypothesized, though none of them are currently supported by strong data. The first hypothesis states that lead binds to ATP or affects cytochrome synthesis, thereby inhibiting cell energy transfer. Another hypothesis is that lead has a negative effect on calcium binding, release or storage, calcium-dependent hormones, or responsiveness of second messengers that rely on calcium. The third hypothesis is that lead depletes home-dependent proteins and cytochromes by hindering cell homeostasis. This can increase the production of free radicals, which may then be neurotoxic (Silbergeld 1992). Although none of these mechanisms are sufficient for explaining the specific neurotoxic effects associated with lead exposure, it has been proven that lead exposure disrupts many cellular biochemical processes and structures critical for brain function, including synaptogenesis, the blood-brain barrier, and second messenger metabolism. (Goldstein 1992).

In children, the exposure to lead causes cognitive function deficits and developmental delays. In a 1974 study by Perino and Ernhart of children with low level lead blood toxicity, the McCarthy scale showed that performance was affected in cognitive, verbal, perceptual, and motor function tests (Silbergeld 1992). Other deficits observed in lead exposed children included attention disorders, decreased auditory sensitivity, and decreased neuromotor processing speed.

As opposed to the large number of Central Nervous System (CNS) effects in children, the majority of the proven effects in adults occupationally exposed to lead involve the Peripheral Nervous System (PNS). These adults, according to Schottenfeld and Cullen, show signs of headache, depression, irritability, increased fatigue, and decreased libido. They also have problems with visuosensorimotor coordination, fine motor control, and somatosensory function, which is impaired through a slowing of the motor nerve conduction velocity (Silbergeld 1992). Additional effects of low-level lead toxicity in adults include increased blood pressure, and in cases of high exposure, renal hypertension (Schwartz 1992).

Because adverse effects can result from both high and low-level lead exposure, it is important to prevent all levels of lead from entering diets by ensuring that lead levels remain low in food products such as maple syrup.

Sources of Lead in Maple Syrup

The majority of lead found in maple syrup does not come from the maple tree itself, but from the lead-soldered tools and containers used in the conversion of the maple sap into syrup. Usually, it is not new equipment that leads to this ingestion, but older models of spouts, buckets, evaporators, pans, and tanks that have not been replaced. For example, in tests involving a number of models, all older metal spouts used to tap the maple trees added lead to maple syrup. However, this was not the case for the newer spouts.

According to the Proctor Maple Research Center, lead can be leached into sap as early as the first stage of maple syrup production and, from there, continue to contaminate throughout the remaining steps. Old tin buckets that are used in the collection process were found to leach lead into the sap, especially if the sap was being collected at a slower rate and had to sit in the containers for large amounts of time. Therefore, the slower the sap runs from the trees, the more lead may be found in the maple syrup. In addition, sap can acquire lead from galvanized and lead-soldered evaporators and storage tanks.

Other sources for lead in maple syrup are the bronze used in the manufacture of gear pumps, old lead-soldered metal spouts used to store the syrup, and dust and dirt containing traces of lead that blows into the sap collection containers (Proctor Maple Research Center 2001). In addition, because approximately forty gallons of sap are evaporated to produce one gallon of syrup containing 2.5% of the original water, the lead content of the process increases the sugar content approximately 66%. This produces syrup’s sweet taste but also concentrates every trace element originally present. Therefore, the concentration of lead, one of these trace elements, is significantly elevated, sometimes up to forty times of that found in the environment.

Graphite Furnace Atomic Absorption Spectrometer (GFAAS)

Chemical Theory

An atomic absorption spectrometer (AAS) uses the absorption of light to measure the concentration of gas-phase atoms. In this experiment, the AAS was used to determine the concentration of the metal lead (Pb) in a liquid sample of maple syrup (Tissue 2000). Metals that exist in their elemental form absorb ultraviolet light when excited by heat, and each metal absorbs a characteristic wavelength. The atomic absorption of discrete wavelengths is the fundamental principle of quantum physics forming the basis for AAS. Because each element has a specific wavelength of absorption, the AAS instrument can look for a specific metal in a sample by selecting a beam of light that corresponds to the specific wavelength of absorption for the desired element (Atomic Absorption Spectroscopy 1998).

The GFAAS used in this experiment was a Perkin-Elmer model 3110 Atomic Absorption Spectrometer with an AS-60 autosampler for furnace analyses. A hollow cathode ray tube containing lead, which preferentially absorbs and emits light at a wavelength of 283.3 nanometers (corresponding to the electronic transition between lead’s two lowest energy states), was used with a standard monochromator, detector, display, atomizer, and light source.

The 5 Stage Process of GFAAS

Once the sample and matrix modifier, a substance added to increase the similarities between standards and samples, have been injected into the graphite tube (4 parts sample: 1 part matrix), a five-step, time and temperature dependent process is used to optimize the results. The first step is a drying phase. During this phase, a temperature of 200 °C is used to dry the sample by removing low boiling point liquids. Argon gas is then used to purge the vapors from the sample. The second stage is a heating stage; the graphite tube temperature is increased to 750 °C. This is a thermal pretreatment of the matrix. Matrix vapors are again flushed out of the tube using inert argon gas. The removal of matrix vapors reduces chemical interference in the final absorption readings. The third stage is a cooling phase, in which the temperature is decreased to 20 °C. The cooling stage precedes the atomization stage because during the atomization it is necessary to induce an extremely large change in temperature in a very short time. The temperature is increased to 1800 °C, and the atomization process produces free ground state atoms. The argon gas is then shut off, allowing the free atoms to remain in the light beam for several tenths of a second. The final stage is a cleaning stage, in which the temperature is raised to 2600 °C in order to remove any remaining residue (Perkin Elmer 1993).

Methods

Machine Preparation

The lamp current on the Graphite Furnace Absorption Spectrometer was set to 15 Amps. Argon gas at 50 psi was used as the purge gas, and a 0.1 M solution of nitric acid was used to rinse the autosampler after each run. The autosampler was set to deliver 25 μL of test
A read delay of 0 seconds was used along with an integration time of 5 seconds.

**Standards Preparation**

Following the 1994 Miller-Ihli article “Graphite Furnace Atomic Absorption method for the Determination of Lead in Sugars and Syrups” that used four standards of 10 ppb, 25 ppb, 50 ppb, and 100 ppb, this experiment utilized standards of equivalent concentrations of lead. A 1.000 ppm 2% nitric acid solution of lead was used to make all standards. A 20 ppm solution of lead was made by adding 49.00 mL of water to 1.000 mL of the 1.000 ppm solution of lead. Then, 0.25 mL of 20 ppm solution were added to 49.75 mL of water to make a 25 ppb solution; 25.00 mL of 100 ppb solution were added to 25.00 mL of water to make a 50 ppb solution; 25.00 mL of 50 ppb were added to 25.00 mL of water to make a 25 ppb solution, and 20.00 mL of 25 ppb were added to 30.00 mL of water to make a 10 ppb solution. The four standards were run in the GFAAS in order to measure their absorbances. These values were then plotted to derive a standard curve.

**Matrix Modifier Preparation**

The matrix modifier solution consisted of a 2% matrix modifier of magnesium nitrate (Mg(NO₃)₂) made by adding 50 mL of water to 0.6 g of magnesium nitrate. Then, 5 μL of matrix modifier and 20 μL of standard or sample were injected into the GFAAS. Even though many different matrix modifiers can be used with Atomic Absorption, magnesium nitrate was used as in Miller-Ihli 1994, which used 0.06 mg magnesium nitrate per sample. 50 mL of matrix modifier were made using the calculation: (6 x 10^-2 g / .005 mL) x 50 mL = 0.6 g magnesium nitrate in 50 mL of water.

**Lead Concentrations in Maple Syrup**

Maple syrup was composed of primarily organic compounds and a few trace elements, including heavy metals such as lead. Due to the nature of GFAAS, which vaporizes samples in order to analyze the heavy metal of interest (in this case lead), all organic matter must be removed from the sample before successful analysis can be carried out.

The first step of the syrup digestion was to vortex the samples in order to attain homogeneity. A 5% solution of sub-boiling HNO₃ was added to each sample, all of which were then heated in a 90-95°C water bath. The samples were heated until all brown vapors had dissipated and the solutions had lost their rust-colored tint and appeared light yellow in color. This step was carried out in order to hydrolyze the carbohydrates contained in the syrup and thus remove the organic content of the sample. Two aliquots of 50% hydrogen peroxide were then added with heating in order to neutralize the nitric acid. Any organic matter that was present in the original sample should have been completely removed in these steps. The samples had thus been made ready for analysis by atomic absorption spectrometry.

**Results**

The absorbances of the four standard solutions were found using the GFAAS. The following data represent the standard curve used for the analysis of the syrup samples.

**Table 2: Standard Values**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance (abs-sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppb</td>
<td>0.624</td>
</tr>
<tr>
<td>50 ppb</td>
<td>0.317</td>
</tr>
<tr>
<td>25 ppb</td>
<td>0.150</td>
</tr>
<tr>
<td>10 ppb</td>
<td>0.020</td>
</tr>
</tbody>
</table>

The mean values of the absorbances were then graphed against the concentrations of the standards to produce the standard curve.

**Table 4: Absorbance of Hannah and John Narowski's Maplestone Farm**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (diluted): 11.73 ppb Actual Concentration: 85.33</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.066</td>
</tr>
<tr>
<td>2</td>
<td>0.088</td>
</tr>
</tbody>
</table>

The mean absorbance was 0.042 abs-sec, which correlated to a diluted concentration of 11.99 ppb. After multiplying by the dilution factor, the actual concentration was found to be 79.93 ppb. This is 170.07 ppb below the Vermont target level of 250 ppb.

**Table 5: Absorbance of Fred Smith's Sugar House**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (diluted): 16.76 ppb Actual Concentration: 111.73 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.039</td>
</tr>
<tr>
<td>2</td>
<td>0.066</td>
</tr>
</tbody>
</table>

A mean value of 0.0475 abs-sec was found for the sample from Fred Smith's Sugar House. This gives a diluted concentration of 12.80 ppb and an actual concentration of 85.33 ppb. This is 164.67 ppb below the target level.

**Table 6: Absorbance of New Morning Farm**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (diluted): 8.70 ppb Actual Concentration: 58.00 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.031</td>
</tr>
</tbody>
</table>

A mean of 0.0475 abs-sec was found for the sample from Fred Smith's Sugar House. This gives a diluted concentration of 12.80 ppb and an actual concentration of 85.33 ppb. This is 164.67 ppb below the target level.
New Morning Farm produced the lowest concentration of lead in its maple syrup. The mean absorbance of 0.020 gave a diluted concentration of 8.70 ppb. This is equal to an actual value of 58.0 ppb, which is 192 ppb below the target level.

The relative concentrations of lead in each syrup sample. Maplestone Farm had the highest lead concentration at 111.73 ppb, and New Morning Farm had the lowest at 58 ppb.

The difference between the Vermont target level of 250 ppb and the lead concentrations found for each sample. All of the tested samples were below the target level.

**Results Analysis**

All syrup samples tested were well below the Vermont State lead concentration target level. Maplestone Farm produced the highest lead concentration at 111.73 ppb. This may be a result of a higher amount of lead in the machinery used to make the syrup. The results found in this experiment show reasonable concentrations of lead. The two sets of data used in this experiment gave results with very little difference between them, so concentrations are believed to be accurate.

In past experiments, higher concentrations of lead were found. The lower concentrations found in this study are probably due to improved processing of maple syrup in Vermont, which has a 250 ppb target level as opposed to the higher EPA target level of 350 ppb. If more research is done on the particular farms and their machinery, additional conclusions could be made as to the causes of the differences in lead concentrations between samples.

**Conclusions**

**Error Analysis**

When working in units of parts per billion, errors are likely to occur, especially during the preparation of standards. Solutions are made using very small amounts of a known concentrations of lead mixed with large amounts of water. Although solutions are vortexed, uniform mixing is difficult to achieve. Errors may also occur due to machine operation. The autosampler injects 25 µL into a very small hole in the graphite tube. The sample may not be completely injected into the hole, and splashing may occur within the graphite tube, causing readings to be either lowered or raised.

**REFERENCES**


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