Leaching of Heavy Metals from Water Bottle Components into the Drinking Water of Rodents

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Providing high-quality, uncontaminated drinking water is an essential component of rodent husbandry. Acidification of drinking water is a common technique to control microbial growth but is not a benign treatment. In addition to its potential biologic effects, acidified water might interact with the water-delivery system, leading to the leaching of heavy metals into the drinking water. The goal of the current study was to evaluate the effects of water acidification and autoclaving on water-bottle assemblies. The individual components of the system (stainless-steel sipper tubes, rubber stoppers, neoprene stoppers, and polysulfone water bottles) were acid-digested and analyzed for cadmium, chromium, copper, iron, lead, magnesium, manganese, selenium, and zinc to quantify the metal composition of each material. In addition the amounts of these metals that leached into tap and acidified water with and without autoclaving were quantified after 1 wk of contact time. On a weight basis, sipper tubes contained the largest quantities of all metals except magnesium and zinc, which were greatest in the neoprene stoppers. Except for cadmium and selenium, all metals had leached into the water after 1 wk, especially under the acidified condition. The quantities of copper, lead, and zinc that leached into the drinking water were the most noteworthy, because the resulting concentrations had the potential to confound animal experiments. On the basis of these findings, we suggest that water-quality monitoring programs include heavy metal analysis at the level of water delivery to animals.

Abbreviation: ICP, inductively coupled plasma.

Drinking water quality is an important factor in experimental outcomes and in the daily care and maintenance of laboratory animals. Animal Welfare Regulations1 and the Guide for the Care and Use of Laboratory Animals26 both stipulate that research animals must be provided clean, potable water, but typically water is only monitored for microbiologic contaminants in the academic environment.21,31,34 Good Laboratory Practice regulations require that animal drinking water be tested for potential confounding contaminants but do not identify specific agents.20 The literature supports the use of acidified water as a means to control bacterial contamination of rodent drinking water, but this practice has several biologic effects, and evidence suggests that it can affect the water-delivery system.28,33,46,49 Water acidification has been reported to have multiple biologic effects.28 When male CD1 mice ingested water acidified to pH 2.0, they had decreased water consumption, decreased weight gain, and decreased numbers of bacterial species in the terminal ileum.22 Rats provided water acidified to pH 2.0 for 24 wk had extensive corrosion of both the enamel and dentin of the first 2 mandibular molars.27,32,50 Mice provided water acidified to pH 2.0 for 120 d had reduced reticuloendothelial clearance rates, spleen weights, and spleen:body weight ratios, indicating a potential for alteration of the immune response.24 Although these effects were attributed to the acidification of the water, they may have been potentiated by the effects of acidified water on the water-delivery system and the subsequent ingestion of leached metals.

In addition, acidifying drinking water can cause metals to leach from water-bottle stoppers. In one study, acidified–deionized water leached more metals from polymer stoppers than did nonacidified–deionized water; the authors suggested that other types of stoppers would be more appropriate for specific nutritional and toxicological studies.28 Although 4 different types of polymer stoppers (rubber, neoprene, vinyl, and silicone) were evaluated, the study had several limitations that were never further examined.28 For example, metal analysis was performed on only 2 of the stopper types (rubber and neoprene), and the authors evaluated only 2 samples of each.28 Furthermore, the metal analysis of the stoppers was limited to 5 metals and the water analysis to 7 metals, with lead being the most notable metal that was absent from the analysis. Moreover, the study28 did not assess the effects of autoclaving on the water-delivery system, nor did the authors perform metal analysis of sipper tubes or the water bottle. As part of our own facility water-quality assurance program, we recently extended our analysis to include point-of-delivery (water bottle) assessment in addition to source assessment for both heavy-metal and microbiologic contaminants. As a result, the goals of the current study were to identify potential heavy-metal contaminants in the water-bottle assemblies and to evaluate the leaching of those metals into the drinking water by using inductively coupled plasma (ICP) spectrophotometry. Specifically, we evaluated neoprene and rubber water-bottle stoppers, stainless-steel sipper tubes, and polysulfone water bottles for contaminant heavy metals after acid digestion. We then evaluated the effects of water acidification and autoclave treatment on the leaching of heavy metals from the water-bottle assemblies into the drinking water after 1 wk of inversion on an empty rodent cage.

Materials and Methods

Acid digestion of materials. The individual components of a water bottle were digested in 1 N trace-metal HCl (Sigma,
Water-bottle components and controls are reported in Figure 1. Slight discrepancies were noted between studies, in particular water bottles in the current study compared with the previous study. We also identified quantifiable amounts of lead, cadmium, chromium, and selenium from neoprene stoppers, compared with previous studies. Neoprene stoppers had less copper, iron, and magnesium but comparable amounts of manganese and zinc per gram of polymer; rubber stoppers in the current study had less copper, iron, magnesium, and zinc per gram of polymer. The stainless-steel sipper tubes had large quantities of all tested metals, which were significantly greater than those in the stoppers for all metals except magnesium and zinc.

Leaching. As determined by ICP spectroscopy, heavy metals leached into the water; the pH for each combination of stopper and water treatment (acid and autoclave) are reported in Table 1. There was no detectable leaching of either cadmium or selenium into the water from any water bottle combination. Acidification and stopper type had significant ($P < 0.05$) main effects on the leaching of copper, iron, zinc, and lead, as did the interaction between acidification and stopper type. Acidification was the only variable to have a significant ($P < 0.05$) effect on chromium. There were significant ($P < 0.05$) 3-way interactions between acidification, stopper type, and autoclaving on both manganese and magnesium, and each individual variable had significant ($P < 0.05$) main effects. Acidified water samples had a pH of 2.23 ± 0.03; tap water samples had a pH of 7.89 ± 0.02. The main effects of acidification, stopper type, and autoclaving; the interaction between acidification and autoclaving; and the interaction between acidification and stopper type all had significant ($P < 0.05$) effects on pH.

Discussion

This study is the first to report the metal analysis for all of components of a water-bottle assembly. Although we used a very similar analysis method (ICP spectroscopy) to that used previously (atomic absorption spectroscopy), the composition of the water-bottle stoppers differed slightly between studies. Similarly, acidified water leached more copper and magnesium than water leached in the current study compared with the previous study. Other differences between studies may be partially responsible for these discrepancies. In particular, water bottles in the current study were left inverted for 7 d instead of 6 d; deionized water was not used; pH was 0.3 units lower; and the source of the stoppers was different.
The results of the current study and those obtained previously\(^2\) collectively suggest that water should be analyzed for contaminants at the level of delivery to animals. These studies further suggest that all materials used in water bottle assemblies should be analyzed for potential heavy-metal contaminants.\(^2\)

The differences in the metal content of the stopper types between the 2 studies highlights the potential variability in stopper materials due to differences in sources and manufacturing processes. To address the variability in material contents, facilities should periodically test these materials for potential contaminants. This suggestion is further supported by the recent reports of lead and melamine contaminants in children’s toys and pet foods from foreign suppliers.\(^8\),\(^10\),\(^11\),\(^47\),\(^52\)

Heavy-metal leaching into drinking water may be problematic due to the individual biologic effects of these components after exposure. Lead has been documented to cause a variety of toxic effects and, as a result, the Environmental Protection Agency has set the ideal water concentration of lead at 0 ppm and the level of action at 0.015 ppm.\(^21\) In the current study, lead leached from neoprene stoppers into acidified drinking water. These lead levels reached 0.2 ppm, thus exceeding the human guidelines.\(^21\) The primary toxic effects of lead are immunologic and reproductive in nature, but lead also can affect other body systems in rodents when administered at high concentrations.\(^7\),\(^9\),\(^13\),\(^14\),\(^23\),\(^39\)-\(^42\),\(^45\),\(^53\),\(^54\) Contaminant concentrations of dietary lead (0.4 μg/g wet weight of feed) have been associated with increased mortality rates in aged male CD1 mice.\(^44\) The amount of lead that these previous mice\(^44\) ingested over the course of their lives was comparable to the exposure (on a μg/g body weight basis) with that which theoretically would be ingested from acidified water in contact with neoprene stoppers. This finding suggests that animals on aging studies should not be
Table 1. Heavy-metal levels in drinking water after 1 wk of contact time with water-bottle components

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stopper</th>
<th>Water</th>
<th>Autoclaved?</th>
<th>Cd  (mean ± SE; n = 5)</th>
<th>Cr  (mean ± SE; n = 5)</th>
<th>Cu  (mean ± SE; n = 5)</th>
<th>Fe  (mean ± SE; n = 5)</th>
<th>Pb  (mean ± SE; n = 5)</th>
<th>Mg  (mean ± SE; n = 5)</th>
<th>Mn  (mean ± SE; n = 5)</th>
<th>Se  (mean ± SE; n = 5)</th>
<th>Zn  (mean ± SE; n = 5)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubber</td>
<td>Acid</td>
<td>No</td>
<td></td>
<td>&lt;0.002</td>
<td>0.015 ± 0.01</td>
<td>0.022 ± 0.008</td>
<td>0.095 ± 0.035</td>
<td>&lt;0.02</td>
<td>12.10 ± 0.18</td>
<td>0.001</td>
<td>&lt;0.05</td>
<td>0.901 ± 0.056</td>
<td>2.15 ± 0.17</td>
</tr>
<tr>
<td>Rubber</td>
<td>Acid</td>
<td>Yes</td>
<td></td>
<td>&lt;0.002</td>
<td>0.009 ± 0.003</td>
<td>0.005</td>
<td>0.053 ± 0.013</td>
<td>&lt;0.02</td>
<td>13.26 ± 0.09</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>2.390 ± 0.225</td>
<td>2.29 ± 0.04</td>
</tr>
<tr>
<td>Rubber</td>
<td>Tap</td>
<td>No</td>
<td></td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>0.030b</td>
<td>&lt;0.002</td>
<td>&lt;0.02</td>
<td>11.76 ± 0.04</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>0.119 ± 0.063</td>
<td>7.01 ± 0.17</td>
</tr>
<tr>
<td>Rubber</td>
<td>Tap</td>
<td>Yes</td>
<td></td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>&lt;0.02</td>
<td>12.88 ± 0.12</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>0.051 ± 0.012</td>
<td>8.12 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Neoprene</td>
<td>Acid</td>
<td>No</td>
<td></td>
<td>&lt;0.002</td>
<td>0.016 ± 0.011</td>
<td>3.250 ± 0.612</td>
<td>0.147 ± 0.050</td>
<td>0.196 ± 0.034</td>
<td>13.74 ± 0.19</td>
<td>0.003 ± 0.001</td>
<td>&lt;0.05</td>
<td>6.112 ± 1.823</td>
<td>2.21 ± 0.03</td>
</tr>
<tr>
<td>Neoprene</td>
<td>Acid</td>
<td>Yes</td>
<td></td>
<td>&lt;0.002</td>
<td>0.013 ± 0.001</td>
<td>3.110 ± 1.253</td>
<td>0.105 ± 0.016</td>
<td>0.240 ± 0.057</td>
<td>14.40 ± 0.10</td>
<td>0.004 ± 0.001</td>
<td>&lt;0.05</td>
<td>6.602 ± 2.474</td>
<td>2.22 ± 0.08</td>
</tr>
<tr>
<td>Neoprene</td>
<td>Tap</td>
<td>No</td>
<td></td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>0.003b</td>
<td>&lt;0.002</td>
<td>&lt;0.02</td>
<td>11.88 ± 0.04</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>0.180 ± 0.005</td>
<td>7.39 ± 0.23</td>
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<tr>
<td>Neoprene</td>
<td>Tap</td>
<td>Yes</td>
<td></td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>&lt;0.02</td>
<td>13.48 ± 0.04</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>0.021 ± 0.003</td>
<td>8.14 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Tap</td>
<td>No</td>
<td></td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
<td>&lt;0.02</td>
<td>12.16 ± 0.09</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>0.003 ± 0.000</td>
<td>8.26 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Acid</td>
<td>No</td>
<td></td>
<td>&lt;0.002</td>
<td>0.004 ± 0.0002</td>
<td>0.007 ± 0.000</td>
<td>&lt;0.02</td>
<td>12.18 ± 0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>0.415 ± 0.002</td>
<td>2.04 ± 0.13</td>
<td></td>
</tr>
</tbody>
</table>

EPA maximal contaminant level 51: 0.005 0.1 1.3 0.03b 0.015 — 0.05b 0.05 5.0b 6.5–8.5b

a Only a single sample had a quantifiable concentration of copper.
b This concentration is the national secondary drinking water regulation. Note that the Environmental Protection Agency (EPA) recommends these secondary standards but does not require compliance with these recommendations.
Manganese and chromium were present in the sipper tubes and neoprene and rubber stoppers. Under all tested conditions, both metals leached at very low levels. Although manganese is a cofactor in several important enzymatic pathways and functions with vitamin K, leading to the formation of prothrombin, there are relatively few studies of toxicity, and deficiency is of a much larger clinical significance.

When administered intraperitoneally to rats at high concentrations (10 mg/kg body weight), manganese causes hyperglycemia and hypoinsulinemia, indicating an effect on carbohydrate metabolism through alterations in insulin release and gluconeogenesis. Chromium is a required element in the diet and is important for normal growth and survival of rodents.

Due to the potential for toxicity, the Environmental Protection Agency has set the limit for chromium at 0.1 ppm for people.

The very small amounts of manganese and chromium that are ingested from the water due to the complex interaction between water systems into acidified water.

**Acknowledgments**

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**References**

Leaching of heavy metals into drinking water


51. United States Environmental Protection Agency. [Internet]. National primary drinking water standards. [Cited 11 April 2011]. Available at: http://water.epa.gov/drink/contaminants/index.cfm#Primary


