Interlaboratory comparison of geosmin and 2-methylisoborneol in municipal tap water

B. Brownlee, C. Marvin, G. MacInnis, M. Charlton and S. Watson

National Water Research Institute, Environment Canada, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada (E-mail: Brian.Brownlee@ec.gc.ca; Chris.Marvin@ec.gc.ca; Gordia.MacInnis@ec.gc.ca; Murray.Charlton@ec.gc.ca; Sue.Watson@ec.gc.ca)

Abstract An interlaboratory comparison (‘round-robin’) for geosmin and 2-methylisoborneol (MIB) was carried out between six laboratories of the Ontario Water Works Research Consortium (OWWRC). Municipal tap water was found to be a suitable medium for distribution of samples. To test stability, geosmin and MIB were added to tap water and stored at 2–4°C. Under these conditions, geosmin concentrations declined by approximately 5% per month for the first 2 months. MIB concentrations were stable over a 158-day period. Three round-robins were carried out individually in 2001, 2003 and 2004. Two levels of geosmin and MIB were used: nominally 10 and 100 ng/l. In 2003 the relative standard deviation for all six participating laboratories were 34, 21, 21 and 22% for low and high level MIB, and low and high level geosmin, respectively. For all but MIB at the low level, there was a marked improvement in agreement between laboratories from 2001 to 2004. However, we recommend use of common analytical standards in order to potentially further reduce interlaboratory variability.

Keywords Geosmin; interlaboratory comparison; methylisoborneol; tap water; taste and odour

Introduction

Lake Ontario is the source of drinking water for several million Ontarians. The northwest corner of the lake is prone to late summer episodes of elevated geosmin concentrations which cause very noticeable earthy odours in the finished water and result in consumer complaints and media attention. These severe episodes (geosmin concentrations >50 ng/l) have been occurring once or twice a decade.

In 1999, a research consortium was established consisting of municipal, provincial and federal agencies, and universities. Currently (2005) the Ontario Water Works Research Consortium (OWWRC) members are: the Regions of Durham, Peel, Halton and Niagara; the Cities of Toronto and Hamilton; Ontario Ministry of the Environment (MOE); National Water Research Institute (Environment Canada); Ontario Clean Water Agency, the overall project manager; University of Waterloo; and Monroe County, NY. Laboratories of six of the participating agencies perform in-house analyses for a range of taste and odor compounds, and employ a variety of analytical methods using both low and high resolution mass spectrometry. Most of the laboratories use solvent extraction with dichloromethane. One laboratory uses resin adsorption.

In order to assess spatial and temporal trends in the occurrence of taste and odor compounds on a regional basis, intercomparability of data from different laboratories is essential. Progress toward determination of causal factors requires differences in quantitative data among the agencies involved in monitoring Lake Ontario source water to be a result of true trends in concentrations of taste and odour compounds, and not differences in analytical methodologies between laboratories. The National Water Research Institute undertook the task of organising an interlaboratory comparison for geosmin (GSM) and 2-methylisoborneol (MIB) in 2001, 2003 and 2004. To the best of our knowledge no
interlaboratory comparisons of this type have been published for geosmin and MIB. In this paper, we present the results of the interlaboratory studies conducted over a 4-year period and some possible factors contributing to interlaboratory variation. The objectives were: (1) to develop a protocol for preparing geosmin and MIB solutions in water that were sufficiently stable to allow time for distribution to, and analysis by, the participating laboratories and (2) by having a common standard, to be able to “harmonise” results from several laboratories. The analytical variables which may contribute to interlaboratory variability were not part of this pilot study.

Methods

A commercially available standard containing 100 mg/l of geosmin and MIB (Wako Chemicals, Richmond, VA) in methanol was added to municipal tap water to give a stock solution with a nominal concentration of 10 μg/l of each. This stock solution was stored at 2–4°C. The stock solution (20 or 200 ml) was added to a large volume (20 l) of tap water, mixed well with a mechanical stirrer, and 1-L subsamples dispensed into pre-cleaned amber glass bottles with PTFE-lined screw caps. Bottles were filled so there was no headspace. Sets of triplicates at each concentration level (10 and 100 ng/l nominally) were distributed to each laboratory. In 2001 and 2004, five laboratories took part, and in 2003 all six laboratories participated. For the 2001 and 2003 round-robin, the municipal water treatment plant was using direct filtration with chlorine as the disinfectant. Just prior to the 2004 round-robin, the water treatment plant implemented several process changes including disinfection with ozone. Chlorine is still used prior to distribution and chlorine levels are in the range 0.9–1.8 mg/l (Halton, 2006). Chlorine levels in the tap water at the laboratory were not determined. In 2004, Burlington tap water averages were: pH 7.57, dissolved organic carbon 1.8 mg/l, alkalinity 82.3 mg/l, and hardness 125 mg/l (from “Halton Averages” in Halton, 2006).

The NWRI method (Brownlee et al., 2004) uses dichloromethane (DCM, 40 ml) for extraction of a 1-L sample with naphthalene-d₈ and biphenyl-d₁₀ as surrogate standards for recovery correction. Extraction is carried out in the sample bottle by shaking on a reciprocal shaker. Extracts are separated, dried and concentrated under an inert gas stream, internal standard (dibenzofuran-d₈) is added, extracts are concentrated to a final volume of 0.2 ml, and analysed by gas chromatography-low resolution mass spectrometry (GC-LRMS). Quantitation ions are m/z 95 for MIB and m/z 112 for geosmin (i.e. the base peaks).

The MOE method (Palmentier et al., 1998) employs addition of the internal standards (deuterated analogs (d₃) of geosmin and MIB) to a 1-L sample upon arrival at the laboratory. The original sample is split into two 500 ml aliquots before addition of Ambersorb 572 resin to each. After adsorption, the resin is collected by filtration, dried and DCM (0.40 ml) added for desorption of analytes followed by analysis using gas chromatography-high resolution mass spectrometry (GC-HRMS) at a resolving power of approximately 7,000. Masses monitored were: m/z 150.1409 for MIB; m/z 153.1597 for MIB-d₃; m/z 112.0888 for geosmin; m/z 115.1076 for geosmin-d₃.

The City of Toronto Laboratory (Lin et al., 1997) uses an 800 ml sample to which MIB-d₃ and geosmin-d₃ have been added as internal standards. DCM (50 ml) is added and extraction is carried out in the sample bottle by rolling. The extract is separated, dried and concentrated on a Zymark Turbo Vap concentrator to a volume of 1 ml and analysed by GC-LRMS. Masses monitored were: m/z 107 for MIB; m/z 110 for MIB-d₃; m/z 112 for geosmin; m/z 115 for geosmin-d₃. Peel Region uses a similar method.

The City of Hamilton Environmental Laboratory uses a sample volume of a minimum of 800 ml which is extracted sequentially with DCM (80, 40 and 40 ml) in 1-L amber
bottles in a tumbler. The extract is separated, dried and reduced to a final volume of 1.0 ml on a rotary evaporator. This is analysed by GC-LRMS by an external standard method. The recovery standard is phenanthrene-d10. Masses used for quantitation are m/z 95 for MIB and m/z 112 for geosmin.

The Durham Regional Laboratory uses a 900 ml sample extracted with multiple portions of DCM (80, 40, and 40 ml) by tumbling for 30, 15 and 15 min, respectively. The separated extract is dried and concentrated on a Zymark Turbo Vap evaporator, and analysed by GC-LRMS using an external standard method. The recovery standard is phenanthrene-d10.

For the storage experiment, twelve 1-L samples, nominally 10 ng/l in geosmin and MIB, were prepared in tap water and analysed by the NWRI method. One set of three samples was analysed the same day (day 0), and the remaining samples stored at 4 °C and analysed in triplicate at 31, 62 and 158 days (Table 1).

Data analysis of the interlaboratory results consisted of calculating the means and standard deviations for results from individual laboratories, as well as the median value for the combined results from all laboratories. These and the one-way analysis of variance were carried out with Microsoft® Excel. Calculation of t values was done using the formulas in Anderson (1987).

Results and discussion
Stability experiments

We received an early indication that geosmin and MIB were quite stable in municipal (chlorinated) tap water through repeat analysis of samples stored for 1 year at 2–4 °C with no headspace. These were tap water samples that had been collected during a period of elevated geosmin levels in the northwest corner of Lake Ontario in August, 1998. The 1998 results are described in Watson et al. (2000): these samples were reanalysed in 1999. In both years a headspace solid phase microextraction method (HSPME) was used (Watson et al., 2000) for analysing these samples. Differences between years were very minor for the most part. For example, samples collected on August 14, 21 and 28 (1998) had geosmin concentrations of 114, 42.4, and 5.9 ng/l, respectively, when analysed in 1998, and 119, 40.4 and 5.8 ng/l, respectively, when analysed in 1999. These results are representative of the whole set of ten samples which were stored and reanalysed. Samples collected on August 21 and 28 (1998) had MIB concentrations of 1.2 and 1.6 ng/l when analysed in 1998, and 1.1 and 1.7 ng/l when analysed in 1999. HSPME was used only in these early experiments and was not used by any of the laboratories for the comparison work, including NWRI. In one study using a natural water, Korth et al. (1992) reported that both natural (−) and racemic geosmin biodegraded rapidly (> 90% in ca. 100 hours) in Murrumbidgee River water at room temperature and normal light conditions. These authors also reported slower biodegradation in samples stored in the cold and dark. The high stability of geosmin and MIB observed in laboratory tap water in the present study was likely due to the original disinfection plus the effect of storage in the cold and dark. Given these indications of the temporal stability of geosmin and MIB in municipal tap water, the results and the implications for water treatment are discussed in the following section.

![Table 1](https://iwaponline.com/wst/article-pdf/55/5/51/439504/51.pdf)

**Table 1** Results of stability/storage experiment for sets of triplicate samples stored at 4 °C in tap water

<table>
<thead>
<tr>
<th>Day</th>
<th>MIB (ng/l)</th>
<th>Geosmin (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.6 ± 0.1</td>
<td>12.7 ± 0.1</td>
</tr>
<tr>
<td>31</td>
<td>9.5 ± 0.1</td>
<td>12.0 ± 0.1</td>
</tr>
<tr>
<td>62</td>
<td>9.6 ± 0.15</td>
<td>11.4 ± 0.1</td>
</tr>
<tr>
<td>158</td>
<td>9.3 ± 0.3</td>
<td>11.3 ± 0.2</td>
</tr>
</tbody>
</table>

*Mean and standard deviation (N = 3)
water stored cold in the dark, this matrix was selected as the distribution medium for the interlaboratory studies.

The results of the stability/storage experiment of 2004 are shown in Table 1. MIB was completely stable, within experimental error, over the entire 158-day period. This was confirmed by a one-way analysis of variance. Geosmin showed a slight decrease in concentration (ca. 5% per month) over the first 2 months of storage. Between-day variation was significantly greater (alpha = 0.05) than within-day variation by a one-way analysis of variance. Pairwise t-testing (alpha = 0.05) showed that Day 0 was significantly different from Day 31 which in turn was different from Day 62 and Day 158. Day 62 was not significantly different from Day 158. However, since the samples were analysed by the laboratories within a few days of receipt, any decline in geosmin concentration during storage would have been a very minor source of variation between laboratories, relative to other factors.

Interlaboratory comparisons
Examples showing the results for 2003 are given in Figures 1–4. Participating laboratories are identified by the number assigned to them in each year. The median values for the combined results are also shown (dashed line). The sets of bars represent the results from the triplicate samples (or, in two cases, duplicates because one sample was lost). In general, interlaboratory differences were greater than intralaboratory differences (Tables 2 and 3). Intralaboratory differences were assessed by looking at the percent relative standard deviation each laboratory had for a set of triplicates. Using MIB at “100 ng/l” as an example, the internal RSDs were: 0.6, 1.4, 3.2, 3.5, 5.4 and 9.6%, i.e. a range of 0.6 to 9.6% for the six laboratories. Corresponding differences from the median value (delta, absolute value) were: 2.1, 12.3, 46.0, 4.0, 5.0 and 3.0 ng/l. Laboratories with the best precision (RSD) did not necessarily have the best accuracy (lowest delta). This is also apparent from Figure 2.

One of the benefits to participating laboratories has been to provide a benchmark, the median value, against which to compare their results. In some cases this has helped to identify operational problems so that corrective steps can be taken. As a group, performance has improved for all but MIB at the low level, as shown by a decreasing relative standard deviation when the data for all laboratories for each year are combined and averaged (Table 3).
Observed differences in data sets between laboratories may have resulted from procurement of geosmin and MIB standards for instrument calibration and recovery correction from different sources, rather than using a common standard for all laboratories. One of the key recommendations of this report is the use of a common analytical standard, or preparation of working standards from a common stock standard, to eliminate this parameter as a potential source of interlaboratory variation. Other factors contributing to interlaboratory variability may have included differences in extraction method, differences in analysis method (e.g. GC-LRMS vs. GC-HRMS), and differences in surrogate standards used for recovery correction and/or quantitation. Use of commercially available deuterated analogues of geosmin and MIB allows use of isotopic dilution as a method of HRMS quantitation. However, use of these deuterated standards is less amenable to conventional GC-MS analysis that is only capable of unit mass resolution. For MIB, the deuterium substitution pattern results in the major fragment ion or base peak (m/z 95) not having the deuterium label. This necessitates the use of a quantitation ion which is less abundant than the m/z 95 ion. As result, the method detection limit when using labelled MIB as a surrogate is too high for routine applications.

**Figure 2** High level (100 ng/l) MIB results for 2003

**Figure 3** Low level (10 ng/l) geosmin results for 2003
Laboratory (municipal) tap water was satisfactory as a distribution medium for geosmin and MIB for interlaboratory comparisons. Commercially available solutions of geosmin and MIB in methanol served as the starting point for these aqueous standards. These two compounds were sufficiently stable in the laboratory water over the short duration of distribution of samples to, and analysis by, the participating laboratories. However, we recommend that stability in any particular tap water be confirmed by means of a storage experiment.

Based on the general trend toward decreasing RSDs in interlaboratory variations over the course of 4 years, these round-robin studies have proven effective in providing

---

**Table 2** Summary of intralaboratory results from 2003 using ranges of relative standard deviations (RSD%), and differences between laboratory means and the median values (delta)

<table>
<thead>
<tr>
<th></th>
<th>MIB-Low 10 ng/l</th>
<th>MIB-High 100 ng/l</th>
<th>GSM-Low 10 ng/l</th>
<th>GSM-High 100 ng/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSD(%)</td>
<td>2.0</td>
<td>0.6</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Delta (ng/l)</td>
<td>0.0</td>
<td>2.1</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>46.0</td>
<td>3.8</td>
<td>47.7</td>
</tr>
</tbody>
</table>

**Table 3** Aggregate results by year in ng/L, including mean (Ave.), standard deviation (SD) and percent relative standard deviation (%RSD)

<table>
<thead>
<tr>
<th>Year</th>
<th>MIB-Low 10 ng/l</th>
<th>MIB-High 100 ng/l</th>
<th>GSM-Low 10 ng/l</th>
<th>GSM-High 100 ng/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Ave. 9.7</td>
<td>93.3</td>
<td>15.0</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>SD 3.3</td>
<td>23.8</td>
<td>5.2</td>
<td>44.8</td>
</tr>
<tr>
<td></td>
<td>%RSD 34</td>
<td>25</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>2003</td>
<td>Ave. 10.1</td>
<td>94.0</td>
<td>9.6</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>SD 3.4</td>
<td>20.0</td>
<td>2.0</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>%RSD 34</td>
<td>21</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>2004</td>
<td>Ave. 13.4</td>
<td>105</td>
<td>12.5</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>SD 4.4</td>
<td>15.3</td>
<td>1.7</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>%RSD 32</td>
<td>14.5</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

---

Figure 4 High level (100 ng/l) geosmin results for 2003
participants with the feedback required to effect improvements in their methods. For example in 2003, Laboratory No. 3 had results that were consistently lower than the median value (by as much as 50%). In 2004, this laboratory had results that were within $-11$ to $+2\%$ of the median (results not shown). With the exception of the low MIB samples, percent RSDs for taste and odour compounds in 2004 were one-half to one-third those in 2001 for all laboratories taken as a whole.

**Conclusions**

Interlaboratory comparisons (“round-robin”, “ring tests”) for geosmin and MIB can be carried out using conventionally-treated (chlorinated) municipal tap water as the distribution medium. Stability tests should be carried out prior to the actual interlaboratory comparisons to confirm that geosmin and MIB are sufficiently stable in a particular water during the time taken for distributing samples to the laboratories and for them to complete the analyses. Comparison against a consensus (median) value allows laboratories to identify and correct procedural problems that might otherwise not be detected. For future work, a common MIB/geosmin standard in a suitable organic solvent is also recommended as a quick means for laboratories to check their procedures, especially at the beginning of the analytical “season”.

**Acknowledgements**

We would like to thank the personnel from all of the participating laboratories for their co-operation and patience, especially: Durham Region: Lev Pidwerbesky, Renate Bruni, Ulyana Chychkevych, Sherry Cui, Sanja Lukic and Eve Williams; Peel Region: Nirmatie Balraj; City of Toronto: Margaret Lin, T. Lusterio and C. Clarke; City of Hamilton: Barry Smallbone, Jessie Blake, James Clark and Bill Anderson; MOE: Vince Taguchi and Jaspal Parmar. We are grateful to Environment Canada and the funding municipalities of the OWWRC for financial support, and to OCWA for project management.

**References**


