Speciation of Iodine in High Iodine Groundwater in China Associated with Goitre and Hypothyroidism

Stig Andersen · Haixia Guan · Weiping Teng · Peter Laurberg

Abstract Iodine intake affects the occurrence of disease in a population. Excessive iodine intake may be caused by a high iodine content of drinking water. Tap water in few locations in Europe contains up to 139 μg/L mostly bound to humic substances, probably leaching from marine sediments in the aquifers. Even higher iodine contents have been found in Chinese waters, previously shown to associate with goitre and hypothyroidism. The aims were to elucidate speciation of high iodine groundwater from deep wells in China and to compare with high iodine waters from Europe. Water was sampled from eight wells in five villages along Bohai Bay, China. Macro-molecules and low molecular weight (MW) substances were separated by size exclusion chromatography (high performance liquid chromatography, Superose 12 HR 10/30, buffer 0.1 M Tris, pH 7.0). Organic material was evaluated by A280 and iodine in fractions measured by the Ce/As method after alkaline incineration. Iodine content of well water varied from 135 to 880 μg/L (median 287 μg/L). The amount of organic material in water was low with A280, <1–5 mAU. The chromatographic traces were similar between samples: One peak of iodine eluted around $K_{AV}$ 0.65 corresponding to MW 5 kDa (humic substances) and one peak at $V_{total}$ (iodide/low MW substances). The fraction of iodine in macro-molecules, suggested to be humic substances, varied from 8% to 70% (median 27%). Iodine and peak absorbance were associated ($p$=0.006). In conclusion, iodine in iodine-rich deep well water in northern China may have marine origin and may associate with humic substances, comparable to shallow well iodine-rich water in Europe. High iodine intake from iodine-rich water suggests the cause of endemic goitre and hypothyroidism in some areas in China being iodine.

Keywords Iodine · Humic substances · Drinking water · Nutrition · Deep well water · China
Introduction

Iodine intake level is important for the occurrence of thyroid disorders and even relatively small deviations from the recommended iodine intake level (lower or higher) associate with an increase in risk of disease in the population [1–3]. At high levels of iodine intake, regular iodine toxicity may develop in individuals [4].

Dietary iodine content is decisive for the iodine intake [5, 6]. Water is a ubiquitous component of the diet and groundwater is an important drinking water resource in many countries [7, 8].

Drinking water iodine content exhibits major regional differences [7–11] which associate with differences in both population iodine excretions [6, 7, 10] and the occurrence of thyroid disorders [11–17]. Iodine-rich waters have been documented in some places [11, 18, 19], and iodine-rich drinking waters in China associated with an increased occurrence of goitre [17, 20] and hypothyroidism [12].

Tap water iodine has been studied in relation to subsurface geology [11, 16, 21], and chemical analysis of natural waters demonstrated variable amounts of aquatic humic substances [22–25]. These were found to hold iodine in iodine-rich waters from one area [18], and it was proposed that groundwater iodine suggests concurring humic substances of marine origin. This could be speculated to be more than a local phenomenon as iodine associated with humic substances [26] and may pass through aquifer transformation zone [27] and hence leach into well water. Whilst this was found in shallow groundwaters, data are lacking for deep well waters. Also, similar findings from different continents would argue for the finding being a general phenomenon.

The aim of this investigation was to elucidate the speciation of high iodine groundwater used for drinking waters in an area in China with a high occurrence of goitre and hypothyroidism and to compare iodine-rich drinking water from Chinese deep and Danish shallow wells using identical analytical procedures.

Materials and Methods

Water Samples

Tap water samples were collected from eight deep water wells that supply drinking water to five villages along the Bohai Bay (Fig. 1) in Huanghua County, Hebei Province, China. Wells from these villages were chosen because of previous demonstration of high occurrence of hypothyroidism [12] and goitre [20] as well as high iodine content of drinking water in that area [19]. Iodine-rich natural waters from Skagen in Northern Jutland, Denmark previously described in detail [18] were used as reference.

Procedures and Solutions

Water samples were collected in iodine-free polyethylene containers. Samples were kept in the dark during shipment and in the laboratory at 4°C until analysis.

Procedures were identical to those used in the analysis of shallow Skagen waters described previously [18]. All analyses were performed on the same column in the same laboratory. The analyses were performed in mixed order where Skagen waters were used for comparison.
Drinking waters were analysed using high performance liquid chromatography (HPLC) size exclusion on ÄKTA Purifier™ (Amersham Pharmacia Biotech, Freiburg, Germany) applying a Superose 12 HR 10/30 column (Amersham Pharmacia Biotech, Freiburg, Germany). This is an agarose gel with exclusion limits from 1,000 to 300,000 D (limits stated by supplier), a void volume \( V_0 \) of 8.4 mL (permeation coefficient \( K_{av}=0 \)) and a total permeation volume \( V_{total} \) of 25.0 mL (\( K_{av}=1 \); determined for the column using blue dextrane and glucose). The column was calibrated with globular proteins (LMW Calibration kit, Amersham Pharmacia Biotech, Freiburg, Germany) for molecular weight (MW) determinations according to the log-linear method \[ 28 \] and the relation was 

\[
MW = e^{16.8 - (0.423 \times V_e)}
\]

Five hundred micro-litres of drinking water was added to the column after filtering through a 0.20-\( \mu \)m membrane (Minisart®, Sartorius, Göttingen, Germany) to eliminate particulate matter. Eluent was 0.1 M Tris buffer with pH adjusted to 7.0. Elution flow rate was 1 mL min\(^{-1} \) and pressure was 1.45–1.52 MPa. Absorbance at 280 nm was recorded and effluent was collected in fractions of 1.5 mL. Experiments were carried out at 21°C. Findings were validated by repeated procedures both at pH 9.0 and on high iodine groundwater from Skagen, Denmark.

Identical iodine concentrations were seen before and after filtering water through a 0.20-\( \mu \)m membrane. Also, no iodine was found in iodine-free waters collected in the polyethylene containers used for well water collection.

**Iodine Determination**

Iodine was determined by the Sandell–Kolthoff reaction modified after Wilson and van Zyl \[ 29 \] as described previously \([5, 30]\). The principle is evaporation and alkaline ashing of the sample followed by re-suspension and measurement of iodine by the spectrophotometric detection of the catalytic role of iodine in the reduction of ceric ammonium sulfate in the presence of arsenious acid. For determination of iodine content, a 1.5-mL sample was used giving an analytical sensitivity of 2.0 \( \mu \)g/L. The intra-assay coefficients of variation were 9.2% (interval 2–4 \( \mu \)g/L, \( n=8 \)), 8.7% (interval 5–9 \( \mu \)g/L, \( n=4 \)), 4.2% (interval 10–15 \( \mu \)g/L, \( n=4 \)) and 1.5% (interval 15–50 \( \mu \)g/L, \( n=5 \)). Recovery of added iodine was >95% and not corrected for.
Statistics

Spearman’s rho and linear regression was used to describe the association between iodine and organic matter in drinking water and geography. A $p$ value of less than 0.05 was considered significant.

Results

Figure 1 shows the iodine content of drinking water collected from five villages situated along the Bohai Bay in Huanghua County, Hebei Province, China. All were deep wells with drinking water samples collected at depths from 500 to 700 m. Iodine content increased from north to south (Spearman’s $\rho_{\text{towns}} 0.90$; $p=0.037$) with the lowest content (187 $\mu$g/L; mean of two wells) in Gaotou in the north to the highest iodine content (805 $\mu$g/L; mean of two wells) in drinking water from Qiantang in the south with a median value of 287 $\mu$g/L between the eight wells.

Figure 2 depicts the chromatographic elution pattern of well water from the villages Qiantang and Houtang with the higher iodine content of well water. The pattern was similar in the remaining villages whilst more pronounced in these two villages. Organic matter was detected as UV absorbance at 280 nm. A narrow mono-modal peak was found around $V_{\text{elution}}$ 19 mL ($K_{\text{av}} 0.65$) corresponding to a MW of 5 to 6 kDa. In addition, minor peaks eluted from around $V_{\text{elution}}$ 17 mL to 19 mL ($K_{\text{av}}$ 0.52 to 0.65; MW 15 to 6 kDa). This was about similar size but with a more narrow peak compared to the chromatographic traces of humic substances in groundwater from Skagen, Denmark ($K_{\text{av}}$ 0.51) [18]. Altering pH did not change the elution pattern or $K_{\text{av}}$ of Chinese waters, i.e., the size of the organic matter (Fig. 3), and the elution of iodine in drinking water was consistently in the fractions containing organic matter.

![Fig. 2 HPLC trace of well water used for drinking water from Qiantang (left) and Houtang (right) villages, Huanghua County, Hebei Province, China (upper panels) and iodine in fractions (lower panels). All samples were from deep wells (500–700 m)](image-url)
The iodine content of deep well drinking water increased in southerly direction (Table 1) as did the absorbance, i.e., the content of organic matter (Spearman's rho 0.83; p=0.011). Between 8% and 70% of the iodine was found in fractions from 16.5 through 19 mL ($K_{av}$ 0.52–0.67) corresponding to an iodine content of around 40 to 200 μg/L (median 80 μg/L), except for one sample from Qikou with a total iodine content of 175 μg/L (Table 1). The 8% of iodine in this sample eluted corresponding to substances with a MW between 15 and 5 kDa. In general, the elution of iodine in deep well Chinese water was comparable to the

![HPLC traces](image)

**Fig. 3** HPLC traces of drinking water from a deep well in Qiantang village, Hebei Province China, performed at pH 7.0 (upper panel) and pH 9.0 (lower panel)

<table>
<thead>
<tr>
<th>Village</th>
<th>Well no.a</th>
<th>Peak</th>
<th>Absorbance (mAU)b</th>
<th>$K_{av}$</th>
<th>MW (kDa)</th>
<th>Well water Iodine (μg/L)</th>
<th>%c of iodine in humic substances</th>
<th>μg/L of waterd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qikou</td>
<td>1</td>
<td>0.5</td>
<td>0.65</td>
<td>6.0</td>
<td>175</td>
<td>8</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.5</td>
<td>0.62</td>
<td>7.2</td>
<td>200</td>
<td>43</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Gaotou</td>
<td>3</td>
<td>0.7</td>
<td>0.64</td>
<td>6.3</td>
<td>260</td>
<td>70</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.4</td>
<td>0.61</td>
<td>7.9</td>
<td>135</td>
<td>45</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Zhangjuhe</td>
<td>5</td>
<td>0.3</td>
<td>0.67</td>
<td>5.2</td>
<td>315</td>
<td>13</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Qiantang</td>
<td>6</td>
<td>1.4</td>
<td>0.66</td>
<td>5.6</td>
<td>730</td>
<td>10</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4.1</td>
<td>0.67</td>
<td>5.5</td>
<td>880</td>
<td>20</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>Houtang</td>
<td>8</td>
<td>1.0</td>
<td>0.66</td>
<td>5.4</td>
<td>640</td>
<td>35</td>
<td>224</td>
<td></td>
</tr>
</tbody>
</table>

*a Ordered from north to south  
*b Absorbance maximum of 280 nm, at peak $K_{av}$ 0.6 to 0.7  
%c % of iodine in fractions corresponding to $K_{av}$ 0.5 to 0.7  
*d Iodine in fractions corresponding to $K_{av}$ 0.5 to 0.7
findings in iodine-rich Danish groundwater [18]. The remaining iodine eluted as low molecular weight substances (around $V_{\text{total}}$) (Fig. 2).

Figure 4 illustrates the association between concentration of the total iodine content in deep well drinking water and the peak absorbance recorded at 280 nm. A significant correlation was found (Spearman’s rho 0.86; $p=0.006$) in keeping with the previous finding in tap water with iodine containing humic substances in Denmark [18]. Linear regression model showed that 69% of the iodine could be explained by the absorbance. The regression line was characterised by [iodine = 186 μg/L × UV280 absorbance + 135 μg/L].

**Discussion**

We investigated drinking water sampled from five villages situated along the coastline of Bohai Bay, Huanghua County in Hebei province, China. This area attracts attention because of reports of a high prevalence of goitre [20], a study describing adverse effects of high iodine intake level on serum thyroglobulin [3] and a study showing increased occurrence of hypothyroidism [12].

The iodine content of drinking water has been determined on most continents. Generally, low iodine contents have been found, i.e., in North America [14], South America [31], Africa [32], Europe [9, 18, 33], Asia [34] and Australia [15]. However, markedly higher iodine contents of drinking water were found in a few locations [11, 18, 19]. We previously described a high iodine content in drinking water from shallow wells in Denmark, Europe, found in concurring humic substances of marine origin [18]. The present study investigated drinking water from deep wells in China, with a collection of waters from depths of 500 to 700 m, using the exact same techniques and equipment in the same laboratory in order to allow for comparisons.

We found macro-molecules in deep well waters in China in the same molecular weight range as that of the shallow waters of marine origin in northern Europe using size exclusion chromatograms which provides information on both molecular weight and quantity of macro-molecules [22, 25, 35]. The chromatographic trace presented differently with subtle shoulders prior to a more narrow main peak. This difference in absorbance with similar retention volumes compared to iodine-rich shallow well waters suggests different concentrations of similar size molecules. This could relate to differences in the sources rock and degree of decomposition of the organic matter as the trace characteristics were in keeping with traces generally obtained from aquatic organic matter [22–24, 35].

![Fig. 4](image_url) Correlation between maximum absorbance at peak and the total iodine content of water from deep wells in villages along the coastline of Bohai Bay, China ($r^2 0.69; p=0.006$)
Marine deposits may be rich in iodine because iodine is assimilated by marine organisms with concentration factors above 30,000 in some algae compared to seawater [21, 36]. Our present findings conform to the notion that high iodine in drinking waters indicate concurring marine organic matter at the source rock and add to the previous findings that the marine origin influences tap water not only in shallow wells but also in deep well waters.

The amount of iodine that eluted with the organic matter in deep well water varied between 40 and 225 μg/L of water except for one sample of 14 μg/L. Yet, the iodine content clearly (p=0.006) associated (r=0.86) with the content of organic matter, and 69% of the variation in iodine content of water from the eight wells along the Bohai Bay could be explained by the content of organic matter in the regression model. This supports that the organic matter is important for the amount of iodine in deep well water, similar to the previous finding in shallow waters [18]. This favours that the organic matter is of marine origin in keeping with also the repeated advancing and retreating Bohai Sea.

Variable amounts of iodine (30–92%) eluted corresponding to low molecular weight substances in waters from all eight wells. The present investigation did not produce an explanation for this difference. The Bohai Bay Basin is one of the main oil containing areas of China [37]. It can be speculated that the iodine-rich deep well waters may be contributed by both sedimentary rocks and underground brines. The low molecular weight iodine content might originate from iodine-rich brines in addition to degradation products from iodine-rich sediments with some differences related to variable sources, decomposition and concentration of organic matter. The relatively high explanatory factor in the regression of total iodine content on absorbance favours the explanation that the iodine is derived from an organic source, and the high iodine content favours that this is of marine origin [18, 21].

Excessive iodine intake may result in hypothyroidism and goitre [2, 4], and such were described in this area [12, 17, 20]. A high iodine intake may originate from a high content of iodine in drinking water [38]. An intake of 2-L drinking water per day in, i.e. Qiantang, could contribute an iodine intake of 1.7 mg from drinking water alone. This exceeds the lowest observed adverse effect level for iodine [4], but even less excessive intake levels may associate with an increase in the occurrence of thyroid disease in a population [2]. Considering the different iodine content of tap water in the villages along the Bohai Bay, a survey of these and other Chinese populations may aid the identification of the lowest observed adverse effect level for iodine [3, 4].

No specific qualitative analysis of drinking water macro-molecules was performed. The HPLC elution pattern of the organic matter in Chinese waters corresponded to that of humic substances [22, 24, 35]. Also, high iodine in tap water suggests concurring humic substances derived from a marine source rock [18]. Some humic substances posses goitrogenic properties [1, 39, 40]. When studying the association between the occurrence of goitre and hypothyroidism and high iodine intake from water, it is necessary to take into account the possibility of goitrogenic properties of humic substances in the drinking water. Concurring humic substances may also influence the bio-availability of tap water iodine [38]. Yet, further studies are necessary to evaluate such effects.

The use of well water for drinking water is increasing globally to provide safe and clean drinking water. This may increase the use of iodine-rich waters with organic matter. Also, water treatment is intensified whilst the influence on iodine nutrition is unsettled. Because iodine in humic substances in drinking water is bio-available and influences iodine nutrition [38] and health [2, 3, 12, 20, 41] and because characteristics of humic substances and the influence on iodine in drinking water may differ between aquifers, it is important to maintain focus on iodine in drinking water.
In conclusion, deep well water used for tap water along the Bohai Bay in China had a high iodine content in accordance with a marine origin and may associate with humic substances. High iodine tap water from two different continents showed similarities in iodine content and in organic matter even though the wells differed also in depths. This calls for attention when planning drinking water supply globally. High iodine intake from iodine-rich water suggests the cause of endemic goitre and hypothyroidism in some areas of China being iodine excess.

References