Determination of total vanadium and vanadium(V) in groundwater from Mt. Etna and estimate of daily intake of vanadium(V) through drinking water

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ABSTRACT

Vanadium(V) can be found in natural waters in the form of V(IV) and V(V) species, which have different biological properties and toxicity. The purpose of this study was to determine the concentrations of total V and V(V) in groundwater from the area of Mt. Etna and to assess the estimated daily intake (EDI) of V(V) of adults and children through drinking water. Water was sampled monthly at 21 sites in 2011. Total vanadium was determined by inductively coupled plasma-mass spectrometry (ICP-MS) and speciation by ion chromatography-ICP-MS (IC-ICP-MS). The concentration of V(V) species ranged from 62.8 to 98.9% of total V, with significantly higher concentrations in samples from the S/SW slope of Mt. Etna. The annual mean concentrations of total V exceeded the Italian legal limit of 140 μg/L at four sites on the S/SW slope. In the absence of thresholds for V(V) intake, only the Environmental Protection Agency (EPA) has calculated a reference dose. Children's EDI of V(V) at the sites with the higher V concentrations exceeded EPA thresholds (9 μg/kg/day). In particular, we found in Camporotondo, Mascalucia, Ragalna and San Pietro Clarenza sites children's EDIs of 11, 9.3, 11 and 9.9, respectively. The EDI of V(V) was significantly higher than the literature range (0.09–0.34 μg/kg/day).

Key words | drinking water, total vanadium, vanadium(V), volcanic area, V(V) estimated daily intake

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INTRODUCTION

Vanadium(V) is a natural element belonging to the transitional metal group (5th A Group) of the periodic table. Its average content in the Earth's crust is 0.014% (Minelli et al. 2000; Moskalyk & Alfantazi 2003; Anke 2004) and the average amount found in soil worldwide ranges from 10 to 220 mg/kg (Ovari et al. 2001; Poledniok & Buhl 2003). Vanadium forms chemical compounds with different oxidation states, from −1 to +5, the most common ones in the environment being +4 and +5; as reviewed in several papers, higher states of oxidation involve higher toxicity (WHO 1988).

V(V) exists as the VO_4^{3-} ion, which is chemically identical and biologically indistinguishable from the PO_4^{3-} ion. Thus, in biological systems it can accumulate in bones and teeth, and it inhibits enzymes that process phosphate (PO_4^{3-}) (Crans et al. 2004).

In natural waters vanadium is found as various chemical compounds with oxidation state +4 or +5 (Minelli et al. 2000; Bishayee et al. 2010; Crebelli & Leopardi 2012), whose distribution depends on water pH, the V concentration, reduction-oxidation potential, and the ionic strength of the system (Pettersson et al. 2003; Wang & Sañudo Wilhelmy 2009).

The main anthropogenic inputs of V into the environment are via combustion of fossil fuels such as coal and oil and through a range of industrial processes (Moskalyk & Alfantazi 2003; Panichev et al. 2006; Mejia et al. 2007; Environment Canada 2010). Vanadium is an ubiquitous metal found naturally in all environmental matrices: air, soil, plants and water. Its concentration measured in these is largely influenced by the geographical location: ambient

air from urban locations is characterized by greater atmospheric levels of vanadium than rural sites and it is, furthermore, dependent on the season (ATSDR 2012); in soil it ranges from 3 to 310 µg/g or more in areas polluted by fly ash (WHO 2000); concentration of vanadium in fresh water is considerably higher than that in seawater: in the first case it ranges from 0.2 µg/L to more than 100 µg/L depending on the presence of effluents and leachates from anthropogenic and/or natural sources entering the water table; in seawater vanadium tends to precipitate so it varies from 1 to 3 µg/L (Sepe *et al.* 2003); lastly, groundwater samples from volcanic areas are characterized by high concentrations of V, from 0.05 to 2.47 mg/L (Giammanco *et al.* 1996; Minelli *et al.* 2000; Farias *et al.* 2003; Fiorentino *et al.* 2007; Farnfield *et al.* 2012; Nicolli *et al.* 2012).

A major aquifer in the area of Mt. Etna, an active volcano in Sicily (Italy), provides drinking water to more than 750,000 people and irrigation water for extensive farmed areas. The local population is, therefore, at risk of excessive V exposure. Knowing its concentrations in the area around Mt. Etna could provide useful data to identify the populations at higher risk of excessive exposure.

An epidemiological study of the consequences of V intake through drinking water among the residents in the Mt. Etna area carried out by our group (Fallico *et al.* 1998) found high V concentrations in biological fluids, but excluded adverse effects of those concentrations on heart arrhythmia, renal lithiasis and diabetes.

Despite extensive investigation of the toxicity of pentavalent V (Llobet & Domingo 1984; Domingo 1996; Aragon & Altamirano-Lozano 2001; Poggioli *et al.* 2001; Ivancsits *et al.* 2002), little is known of the effects of high-level exposure on human health, nor have thresholds been set by national or international bodies or institutions such as the European Food Safety Authority (EFSA) or the World Health Organization (WHO). The Environmental Protection Agency (EPA) alone has suggested a reference dose (RfDo) for oral intake of 9×10^{-3} mg/kg/day body weight (bw) based on the observation of decreased hair cysteine levels in rat studies (US-EPA 2013). Research is ongoing to determine whether regulations are needed (US-EPA 2008).

In 1998, the technical-scientific body of Istituto Superiore di Sanità (the Italian Health Ministry's research body) proposed a limit value of 50 µg/L for total V in drinking water, to be applied for a period of 5 years. The value subsequently became the legal threshold (Legislative decree no. 31/2001). However, the high total V concentrations found in the Mt. Etna aquifers led to a derogation for this area (total V, 160 µg/L; pentavalent V, 50 µg/L) in 2004–2011. The current national threshold for total V is 140 µg/L (Ministerial decree of 22 December 2011), whereas there are no limitations for V(V), the more toxic species.

The purpose of this study was to measure the concentrations of total V and V(V) in drinking water collected in 21 small towns in the area of Mt. Etna and to calculate the estimated daily intake (EDI) of V(V) of the local populations through water.

MATERIALS AND METHODS

Sampling and *in situ* sample treatment

Groundwater was sampled monthly throughout 2011 in 21 small towns: Aci Bonaccorsi (1), Aci S. Antonio (2), Adrano (3), Belpasso (4), Bronte (5), Camporotondo Etneo (6), Gravina (7), Mascalucia (8), Nicolosi (9), Pedara (10), Ragalna (11), S. Agata li Battiati (12), S. Giovanni Galermo (13), S Giovanni la Punta (14), S. Gregorio (15), S. Maria di Licodia (16), S. Pietro Clarenza (17), Trecastagni (18), Tremestieri Etneo (19), Valverde (20) and Viagrande (21) (Figure 1); 12 samples per site were collected. Samples for total V determination were acidified *in situ* with 0.5% v/v HNO₃ 65% Suprapur® (Merck, Darmstadt, Germany); for V(V), Na₂EDTA salt was added to obtain a concentration of 25 mM in 50 mL. Samples were refrigerated during transport and stored at 1–5 °C in the laboratory until analysis, within 2 days of sampling.

Principle of the method

Total V was determined with an inductively coupled plasma-mass spectrometry (ICP-MS) Elan DRC-e apparatus (Perkin Elmer Sciex, Norwalk, CT, USA) equipped with a Meinhard quartz concentric nebulizer and quartz cyclonic spray chamber. Vanadium was detected as V^+ ions at m/z = 51 using yttrium ion (Y^+) at m/z = 88 as the internal standard, with external calibration, applying the UNI ENISO 17294-2:2005 method.
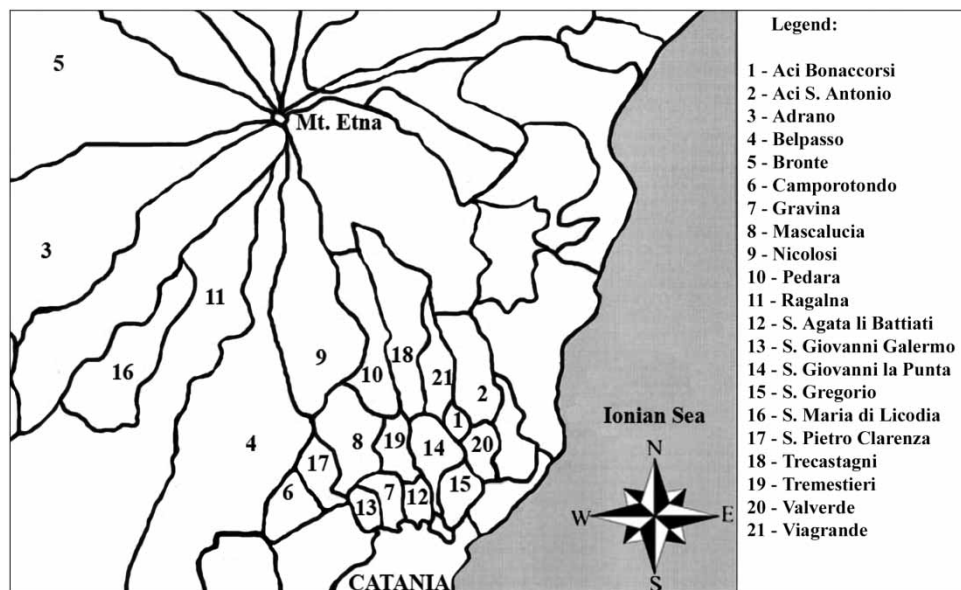
Figure 1 | Location of the small towns in the Mt. Etna area where tap water samples were collected.

The chromatographic apparatus was a Flexar high-performance liquid chromatography (HPLC) system (Perkin Elmer Sciex) equipped with a quaternary pump, on-line vacuum eluent degasser, column oven and autosampler. Chromatographic separation of V complexes was achieved with two Metrosep A Supp 4/5 guard columns in series (Metrohm AG, Herisau, Switzerland) and a polyvinyl alcohol anion-exchange guard column with quaternary ammonium groups connected to an ICP-MS nebulizer through a Rheodyne 6-channel automatic switching valve (IDEX Health & Science, Wertheim-Mondfeld, Germany). Chromatographic data were collected, stored and processed using Perkin Elmer's Chromera Software (v. 2.1).

The V(V) species was converted to $[VO_2 (EDTA)]^{3-}$ by adding Na_2EDTA to the water sample (EDTA is ethylenediaminetetraacetic acid). Under the same conditions, the complex $[VO (EDTA)]^{2-}$ was formed by conversion of the V(IV) species. The two anionic species were separated by a low-capacity anion exchanger using $NaHCO_3/Na_2CO_3/Na_2EDTA$ buffer and detected as V^+ ions at $m/z = 51$ (Aureli *et al.* 2008) by the inductively coupled dynamic reaction cell plasma mass spectrometry (ICP-DRC-MS) apparatus connected to the chromatograph. Although V(IV) quantification was not a goal of the study, it was determined as a potential interfering species.

The approach used for detection limit calculation ($p = 95\%$) used the uncertainty of linear calibration (ISO 1997, 2000), as stated in the following equation:

$$MDL = 2t_{p;v}$$

$$\frac{\sqrt{\frac{\sum_{i=1}^{I}\left(y_{i,\text{meas}} - a - bx_i\right)^2}{I - 2}}}{b} \times \sqrt{\frac{1}{K} + \frac{1}{I} + \frac{\bar{x}^2}{\sum_{i=1}^{I}(x_i - \bar{x})^2}} \quad (1)$$

where $y_{i,\text{meas}} =$ measured intensity of calibration standards; $t_{p;v} = t$-Student value ($p = 95\%$, $v = I - 2$ degrees of freedom); $K =$ number of replicated determination on same sample ($K = 1$); $I =$ number of calibration standard ($I = 8$).

The method's detection limits were 0.5 μg/L and 1.8 μg/L for total V and V(V), respectively.

Chemicals

Ultrapure water (18.2 MΩ·cm), filtered at 0.22 μm, was obtained from a Milli-Q Gradient system (Millipore, Molheim, France). V_2O_5 99.6% and $VOSO_4\cdot H_2O$ 97% (both from Sigma-Aldrich, Milwaukee, WI, USA) were used for speciation analysis as V(V) and V(IV) reference materials,

respectively. Using these salts, a V(IV) standard stock solution of 1,000 mg/L was prepared in water and 1,000 mg/L of V(V) standard stock solution was obtained by dissolving the necessary amount of salt in 0.45 M HNO$_3$ 65% Suprapur® by continuous stirring for 30 min. V$_2$O$_5$ dissolves in acid as VO$_2^+$ according to the following equilibrium (Greenwood & Earnshaw 1984):

$$V_2O_5 + 2H^+ \rightleftharpoons 2VO_2^+ + H_2O \qquad (2)$$

For each species, intermediate standard solutions of different concentrations (from 0.5 mg/L to 10 mg/L) were prepared by diluting the 1,000 mg/L solutions in 25 mM EDTA-Na$_2$ (purity >99% Sigma-Aldrich), thus forming the EDTA complexes. Eight calibration solutions (from 5 μg/L to 300 μg/L) containing both V(IV) and V(V) complexes were prepared in the same way by diluting intermediate standard solutions of appropriate concentrations.

A multi-element certified reference standard containing 100 mg/L V and 1,000 mg/L Y (Merck), the latter used as internal standard, were employed to prepare standards for total V analysis after suitable dilution in 0.5% v/v HNO$_3$ 65% Suprapur®.

Eluent for chromatographic separation was prepared from the salts at a concentration of 6 mM NaHCO$_3$/2 mM Na$_2$CO$_3$/5 mM Na$_2$EDTA (Carlo Erba, Milano, Italy) in ultrapure water then filtered through a 0.45 μm membrane.

All solutions except the total V and Y solutions were freshly prepared before use.

Estimate of daily vanadium intake through drinking water

The EDI of V(V) (μg/kg bw/day) through drinking water was calculated using the following equation (US-EPA 2000):

$$EDI = (IR \times C)/BW \qquad (3)$$

where *IR*, the ingestion rate, is assumed to be 2 L/day for adults and 1 L/day for children; *C* is the metal concentration (μg/L) and *BW* is the body weight assumed to be 70 kg for adults and 16 kg for children (Copat *et al.* 2013).

Statistical analysis

For statistical analysis, samples were divided into two sets based on the springs supplying the towns: A1 samples were supplied totally or partly by Ciapparazzo spring, on the western slope of Mt. Etna (sites 3, 4, 6, 7, 8, 11, 16 and 17), whereas A2 samples were from the towns receiving water from other springs on the S/SE slope (sites 1, 2, 5, 9, 10, 12, 13, 14, 15, 18, 19, 20). Data were subjected to Student's *t*-test for unpaired samples; a *p*-value <0.05 was considered significant.

RESULTS

The annual mean concentration of total V in the water samples from each town ranged significantly from 15.6 μg/L to 182 μg/L (Figure 2), with an expanded uncertainty respectively of 2.7 μg/L and 26 μg/L ($k = 2$; $p = 95\%$).

The samples from sites 3, 4, 7 and 16 exhibited the greatest concentration variability (Figure 3). Furthermore, at sites 3, 4 and 7 the legal limit for total V was exceeded in some samples during the year, but the annual mean was under the legal threshold (Figure 3).

Pentavalent V ranged from 62.8% to 98.9% of total V and the shape of the scatterplot showed that it remained fairly constant at all total V concentrations, which ranged from 15.6 μg/L to 182 μg/L (Figure 4).

The total V concentrations found in area A1 (western slope of Mt. Etna) were higher than those found in area A2 (S/SE slope) and the difference was highly significant ($p < 0.001$) (Figure 5).
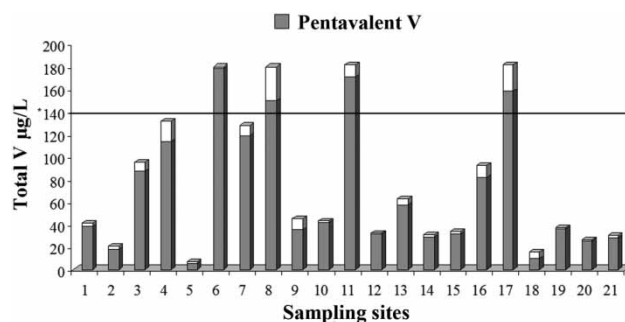

Figure 2 | Annual mean concentration of total V and pentavalent V (μg/L) determined in drinking water samples from 21 towns. *Legal limit value (Ministerial decree of 22 December 2011).
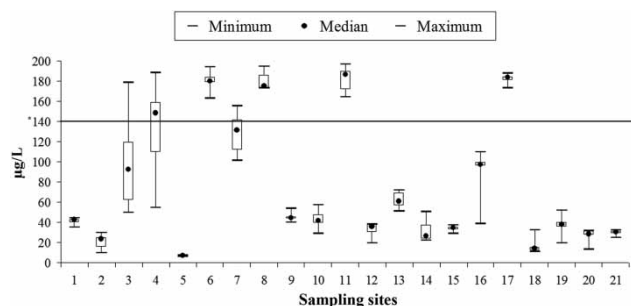
Figure 3 │ Total V box plot (µg/L). *Legal limit value (Ministerial decree of 22 December 2011).
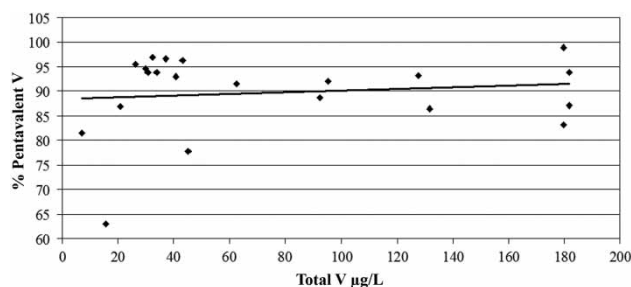

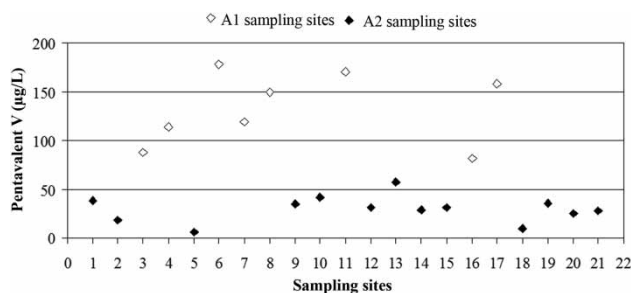**Figure 4** │ Pentavalent V as a proportion of total V.

Figure 5 │ Annual mean concentration of pentavalent V at A1 and A2 sampling sites.

The EDI of V(V) at some A1 sites (6, 8, 11 and 17) exceeded the children's RfDo calculated by EPA.

As shown in Figure 2, the annual mean concentration of total V exceeded the Italian legal limit of 140 µg/L at sites 6, 8, 11 and 17. At the same sites, children's EDI of V(V) also exceeded EPA's RfDo (Table 1).

DISCUSSION

Food is considered the main source of V for the general population, with an EDI of a few tens of micrograms

Table 1 │ EDI of V(V) (µg/kg/body weight/day) in adults and children due to Etna ground water ingestion

N	Countries	EDI in adult	EDI in children
1	Aci Bonaccorsi	1.1	2.4
2	Aci S. Antonio	0.5	1.1
3	Adrano	2.5	5.5
4	Belpasso	3.3	7.1
5	Bronte	0.2	0.4
6	Camporotondo	5.1	11[a]
7	Gravina	3.4	7.4
8	Mascalucia	4.3	9.3[a]
9	Nicolosi	1.0	2.2
10	Pedara	1.2	2.6
11	Ragalna	4.9	11[a]
12	S. Agata li Battiati	0.9	2.0
13	S. Giovanni Galermo	1.6	3.6
14	S. Giovanni la Punta	0.8	1.8
15	S. Gregorio	0.9	2.0
16	S. Maria di Licodia	2.3	5.1
17	S. Pietro Clarenza	4.5	9.9[a]
18	Trecastagni	0.4	0.9
19	Tremestieri Etneo	1.0	2.3
20	Valverde	0.7	1.6
21	Viagrande	0.8	1.8

[a]EDI > RfDo

(WHO 1996). Studies conducted on the population of Tarragona (Spain) considering a variety of food items disclosed EDI values of V of 28.9 µg/day (Bocio *et al*. 2005) and 156 µg/day (Domingo *et al*. 2012), the equivalent of 0.41–2.2 µg/kg/day in an individual weighing 70 kg.

Drinking water seems to contribute less, with values typically between 1 and 6 µg/L (Davies & Bennett 1983). In a US study, the V concentration was <10 µg/L (peak concentration, 70 µg/L; mean concentration, 4.3 µg/L) in 91% of tap water samples (Durfor & Becker 1963).

Studies of human exposure to V from drinking water are limited (Lagerkvist *et al*. 1986; USGS 2009; ATSDR 2012). The mean European EDI of V according to EFSA (EFSA 2004) is 10–20 µg/person/day, or 0.2–0.3 µg/kg bw/day, which is at least three orders of magnitude less than the lowest doses reported to cause adverse effects in rats (800 µg/kg bw/day) and humans (about 200 µg/kg bw/day)

(EFSA 2004). An EDI of V of c. 2 μg/day (equivalent to 0.03 μg/70 kg/day) has been estimated based on the assumption of 2 L daily intake of drinking water (ATSDR 2012).

The present findings of a concentration range of 0.4–11 (Table 1) and a median of 2.4 μg/kg bw/day indicate an EDI at least one order of magnitude greater than the one calculated by the US Agency for Toxic Substances and Disease Registry (ATSDR). Exposure depends significantly on geographical location. Notably, high V concentrations have been found in groundwater from volcanic areas (40–100 μg/L) (WHO 1988; Minelli *et al.* 2000; Farias *et al.* 2003). In previous studies (Sciacca *et al.* 1995; Fallico *et al.* 1998) we also found total V concentrations exceeding 100 μg/L (mistakenly reported as mg/L in Fallico *et al.* 1998 due to a clerical error) since at least 1978 in the western Mt. Etna area.

The balance between benefit and risk due to oral intake of V is still unclear.

The biological and physiological effects and the potential toxicity of V have been extensively investigated. Although poorly absorbed in the gastrointestinal tract, V is rapidly distributed through the circulation; concentrations initially peak in kidney, liver and lungs, while bone and muscle are long-term storage sites (Aragon & Altamirano-Lozano 2001; Ivancsits *et al.* 2002). Vanadium is an essential element for normal cell growth in trace amounts. It enhances glucose transport and metabolism in different tissues and cell types; it promotes glycogen synthesis (Tamura *et al.* 1984) and lipogenesis (Shisheva & Shechter 1992); it inhibits lipolysis (Shisheva & Shechter 1992) and gluconeogenesis (Tolman *et al.* 1979). Vanadium affects a variety of enzyme systems including phosphatases, ATPases, peroxidases, ribonucleases, protein kinases and oxidoreductases. Multiple biochemical and molecular actions seem to be involved in its inhibitory effects on various tumour cells of human origin (Bishayee *et al.* 2010). Vanadium compounds enhance insulin sensitivity and glucose homeostasis in animal models of type 1 and 2 diabetes mellitus and in a small number of human subjects with diabetes (Elberg *et al.* 1994; Cohen *et al.* 1995; Goldfine *et al.* 2000; Cusi *et al.* 2001). They have also been shown to exert neuroprotective effects (Kawano *et al.* 2001), for instance, protecting diabetic rats from focal cerebral ischaemia (Liu *et al.* 2012). Inorganic V and vanadium compounds also have a wide range of cardioprotective effects in myocardial ischaemia/reperfusion-induced injury, myocardial hypertrophy, hypertension and vascular diseases; furthermore, administration of V compounds improves cardiac performance and smooth muscle cell contractility, and modulates blood pressure in various models of hypertension (Bhuiyan & Fukunaga 2009).

An epidemiological study conducted by our group in the same area as the present investigation (Fallico *et al.* 1998) sought a possible correlation between total V concentration in blood and urine and heart arrhythmia, renal lithiasis, diabetes and arterial hypertension. Interestingly, concentrations were higher among controls than among cases ($p > 0.01$) with an odds ratio of 0.22 for diabetes, of 0.17 for lithiasis, and of 0.36 for arrhythmia, suggesting a probable protective role for V. No correlation was found for arterial hypertension.

On the other hand, high blood concentrations of V(V) may cause adverse effects in mammals, such as haemopoietic changes, nephrotoxicity, and reproductive and developmental toxicity (Llobet & Domingo 1984; Domingo 1996; Aragon & Altamirano-Lozano 2001; Poggioli *et al.* 2001; Ivancsits *et al.* 2002). Since the limited oral carcinogenicity studies performed to date do not allow any conclusion to be drawn, V(V) has been classified as 'possibly carcinogenic to humans' (Group 2B) (IARC 2006). Current findings suggest that V(V) does not act as a classic genotoxic agent, but rather as a weak, local carcinogen exerting effects through secondary (genotoxic or non-genotoxic) mechanisms (Assem & Levy 2009). Both acute and chronic poisoning have been described in V(V)-exposed workers, including respiratory problems (Zhang *et al.* 2009), histological changes, functional damage to the spleen, and effects on the humoral immune response (Pinon-Zarate *et al.* 2008).

The high V concentrations found in groundwater supplies from Mt. Etna suggest that V exposure could also correlate with some pathological conditions. Pellegriti *et al.* (2009) assessed the incidence of thyroid cancer in a Sicilian population and found that residents in Catania province, where Mt. Etna is located, appear to have the highest incidence of papillary thyroid cancer in Sicily and a higher incidence compared with other volcanic areas of the world such as Hawaii, the Philippines and Iceland (Kung *et al.* 1981; Arnbjornsson *et al.* 1986; Duntas & Doumas 2009). Since other environmental factors associated with

volcanic areas cannot be excluded as risk factors for thyroid cancer, an effect of the presence of V may be hypothesized.

CONCLUSIONS

If areas at risk of excessive V exposure were to be defined on the basis of V concentrations in groundwater, they would be the small towns supplied wholly or partially by Ciapparazzo spring (area A1). Furthermore, here we found high levels of V(V), known for its potentially significant effects on human health. In the area examined in the present study, the amount of V associated with drinking water and consequently the EDI of V(V) were significantly higher than the literature, ranging from 0.09 to 0.34 μg/kg/day in adults (ATSDR 2012). The resulting EDI could be useful for future evaluations, when more exhaustive toxicological data on V(V) become available.

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