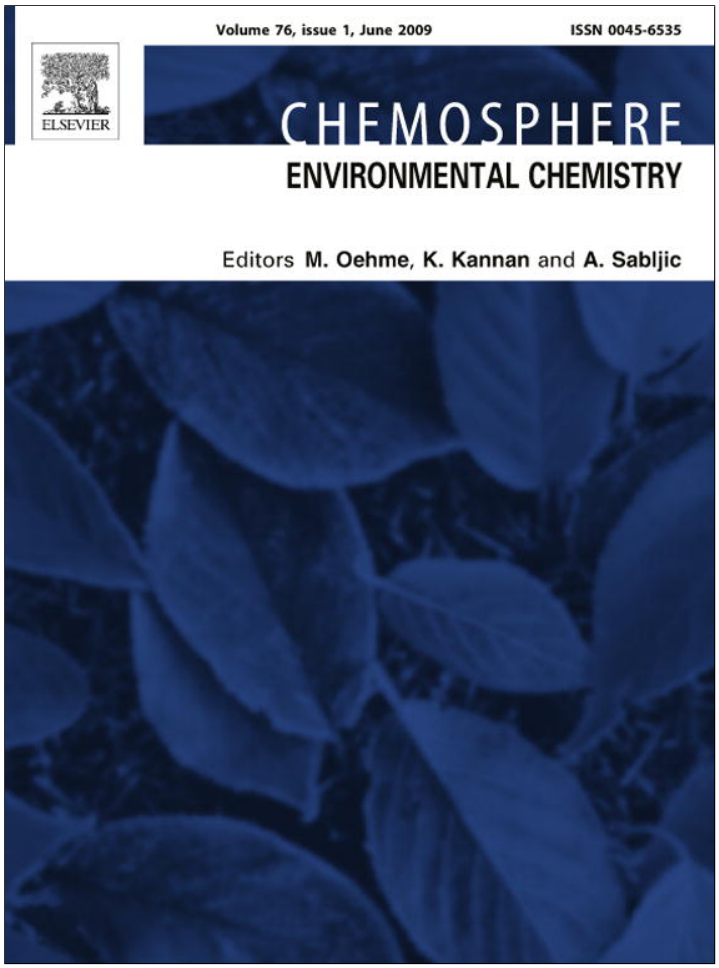

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Occurrence of perchlorate in drinking water, groundwater, surface water and human saliva from India

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ABSTRACT

Perchlorate (ClO_4^-), which is used as an oxidizer in jet and rocket fuels, pyrotechnic devices and explosives, is a widespread contaminant in surface waters and groundwater of many countries. Perchlorate is known to affect thyroid function. Despite the compound's widespread occurrence and potential health effects, perchlorate levels in drinking water in India are not known. In this study, water samples collected from 13 locations in six states ($n = 66$), and saliva samples collected from four locations in three states ($n = 74$) in India, were analyzed for perchlorate using high performance liquid chromatography interfaced with tandem mass spectrometry (HPLC–MS/MS). Perchlorate was detected in most (76%) of the water samples analyzed at concentrations above the quantitation limit of 0.02 μg L^{-1}; concentrations ranged from <0.02 to 6.9 μg L^{-1} (mean: 0.42 ± 1.1 μg L^{-1}; median: 0.07 μg L^{-1}). Mean concentrations of perchlorate in drinking water, groundwater, bottled water, surface water and rain water were 0.1, 1.0, <0.02, 0.05 and <0.02 μg L^{-1}, respectively. From a total of 66 water samples analyzed, only three samples contained perchlorate levels above 1 μg L^{-1}; all three were groundwater samples. Perchlorate was found in the saliva samples analyzed at concentrations above 0.2 μg L^{-1} and up to 4.7 μg L^{-1} (mean: 1.3 ± 1.3 μg L^{-1}; median: 0.91 μg L^{-1}). No remarkable differences in perchlorate concentrations were found among the sampling locations of water or saliva or in subgroups stratified by gender or age. Perchlorate concentrations in water samples from India are one to two orders of magnitude lower than the concentrations reported for the United States.

1. Introduction

Perchlorate is used as an oxidant in rocket fuel, missiles, flares and fireworks (Urbansky, 1998). Large scale production of perchlorate began in the 1940s. Natural sources of perchlorate have also been reported, such as in sodium nitrate deposits in Chile (Urbansky et al., 2001). In addition, it is thought that atmospheric processes can produce perchlorate (Dasgupta et al., 2005). Perchlorate is readily soluble in water (solubility in the range of tens to hundreds of g per liter), mobile in aquatic systems and does persist for many decades under typical groundwater and surface water conditions (Urbansky, 1998; Gal et al., 2008). Perchlorate is incorporated into foodstuffs such as cows' milk and leafy vegetables, through food chain transfer and accumulation (Kirk et al., 2003, 2005; Sanchez et al., 2005). Drinking of water is a route of exposure of humans to perchlorate. Since 1997, when it was found in groundwater and in some surface waters across the United States, perchlorate has been listed as a contaminant in drinking water monitoring programs (USEPA, 1998).

Perchlorate concentrations in water have been well determined in the United States (USEPA, 2004; Snyder et al., 2005; Parker et al., 2008). The widespread occurrence of perchlorate in drinking water in the United States (Urbansky, 2002) led to concerns regarding perchlorate-induced iodine deficiency, and thereby thyroid hormone level alterations (Crump and Gibbs, 2005). Thyroid hormone (viz., T3 and T4) deficiency during pregnancy along with low iodine intake can adversely affect neurodevelopmental outcome in the fetus (Li et al., 2000). In 2005, the United States Environmental Protection Agency (EPA) established a reference dose for perchlorate of 0.7 μg kg^{-1} bw d^{-1} (USEPA, 2005a). The EPA's interim health advisory level for perchlorate in drinking water is 15 μg L^{-1} (USEPA, 2005a). The Massachusetts Department of Environmental Protection promulgated a 2 μg L^{-1} drinking water standard, whereas the California Department of Health Services proposed 6 μg L^{-1} as the maximum contaminant level for perchlorate in drinking water in that state (USEPA, 2005a). Several states in the United States have set advisory levels of perchlorate in the range of 1–18 μg L^{-1} (USEPA, 2005b). Occurrence of perchlorate in surface waters and drinking water has also been documented in Korea (Quinones et al., 2007), Japan (Kosaka et al., 2007) and Canada (Backus et al., 2005).

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Exposure of humans to perchlorate via foodstuffs and drinking water has been documented (Murray et al., 2008). Urine, breast milk and saliva have previously been used as matrices in biomonitoring of human exposures to perchlorate (Kirk et al., 2005; Blount et al., 2007; Oldi and Kannan, 2009). Assessment of human exposures to perchlorate is important, since this compound blocks iodine uptake in the thyroid gland, which can lead to a decrease in the production of thyroid hormones (T3 and T4) essential for neurodevelopment (Wolff, 1998).

Previous studies on perchlorate levels in environmental samples and biota have been conducted primarily in the United States (Urbansky, 2002). Environmental contamination by perchlorate in other countries has received little attention, even in those countries that have had extensive armament manufacture or perchlorate production facilities (http://www.thehindu.com/2004/02/20/stories/2004022003601200.htm). Prior to our study, there has been no documentation of the occurrence of perchlorate in water or saliva from India. In this study, concentrations of perchlorate were determined in water and saliva samples collected from urban and rural locations in India, to establish baseline values. Water and saliva samples were prepared by a rapid method that involved simple filtration, and were analyzed by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS).

2. Materials and methods

2.1. Sample collection

Water and saliva were sampled in India during August–September 2008. In all, 66 grab samples of drinking (tap) water, groundwater (or well water), bottled water, surface water (rivers and lakes) and rain water were collected from 13 locations in six states (Tamil Nadu, West Bengal, Bihar, Maharashtra, Karnataka and Pondicherry) (Fig. 1). Drinking water samples were collected from restaurants and homes. In this study, the term drinking water denotes water that was distributed through publicly owned treatment facilities (i.e., after water treatment). Groundwater samples were collected from public bore wells or private wells, or from restaurants where groundwater is used (for wash basins) without treatment. Surface water samples were collected from lakes and rivers. Water samples were collected in clean polypropylene tubes. Several brands of bottled water were purchased from grocery stores. Of the 66 samples, 60% were drinking water, 27% were groundwater, 7% were bottled water, 3% were surface water and 3% were rain water.

Saliva samples were collected from adult volunteers in Bengaluru, Chennai, Mettupalayam and Patna (Fig. 1). From a total of 93 samples, only 74 could be analyzed, since the remaining 19 had inadequate volume. Volunteers were asked to spit into polypropylene tubes after rinsing their mouths with bottled water. Volunteers' gender and age information was recorded. Samples were stored at 4 °C until analysis. The New York State Department of Health (NYSDOH) Institutional Review Board (IRB) approved the study protocol for the collection of saliva from adult volunteers.

2.2. Sample preparation

The method for the analysis of perchlorate in water and saliva has been described previously (Oldi and Kannan, 2009). For water samples, approximately 2 mL of sample were filtered through a 0.22 μm nylon filter. Exactly 0.9 mL of filtered sample and 1 ng of ^{18}O-labeled perchlorate internal standard ($Cl^{18}O_4^- > 90\%$; Cam-

Fig. 1. Map of India showing water and saliva sampling locations.

bridge Isotope Laboratories, Inc., Andover, MA) were added to a sample vial. The samples were vortex mixed and analyzed by HPLC–MS/MS.

For saliva, samples were centrifuged for 10 min at 3000*g*. An aliquot of 0.1 mL of the supernatant was transferred into a 15 mL polypropylene tube, and 1 ng of $Cl^{18}O_4^-$ (as internal standard) was added; the final volume was adjusted to 1 mL with deionized water. The deionized water used for standard preparation, mobile-phase preparation and dilutions and was generated with a NANOpure Diamond ultrapure water system (Barnstead International, Dubuque, IA); it had a resistance of 18.2 MΩ cm. Perchlorate was not detected in the deionized water. The sample was then vortex mixed, and filtered through a 0.22 μm nylon filter into a sample vial. Additional clean-up of saliva was recommended to remove large biomolecules (Oldi and Kannan, 2009).

2.3. Instrumental analysis

Separation of perchlorate ion in water and saliva was achieved with a 250 mm × 2 mm IonPac AS-21 anion exchange column (Dionex, Sunnyvale, CA). With use of a 200 mM methylamine mobile phase, perchlorate eluted at 5 min (10 min total run time) and was introduced to Micromass Quattro LC (Waters Corporation, Milford, MA) mass spectrometer operated in the negative electrospray ionization mode. Multiple reaction monitoring allowed the following fragmentation patterns to be observed: *m/z* 99 ($^{35}ClO_4^-$) → *m/z* 83 ($^{35}ClO_3^-$), *m/z* 101 ($^{37}ClO_4^-$) → *m/z* 85 ($^{37}ClO_3^-$) and *m/z* 107 ($^{35}Cl^{18}O_4^-$) → *m/z* 89 ($^{35}Cl^{18}O_3^-$). The peak area ratio of ^{35}Cl:^{37}Cl was used for confirmation of the perchlorate ion in the sample. A ratio of 3.12 ± 25% was considered acceptable. Quantification was based on an isotope-dilution technique. The relative response of native perchlorate to the isotopically labeled internal standard was used for quantification according to the following formula: Relative response = (Area $^{35}ClO_4^-$/Area $Cl^{18}O_3^-$) * [$Cl^{18}O_3^-$].

2.4. Quality assurance and quality control (QA/QC)

A 10-point calibration standard containing perchlorate concentrations ranging from 0.01 to 100 μg L^{-1} was injected with each batch of 20 samples. A continuing calibration check (CCC) was performed after every 10 samples, with a calibration standard to check for instrumental drift. Along with every 20 water or saliva samples, a laboratory reagent blank (LRB), a laboratory fortified blank (LFB) and a laboratory fortified duplicate (LFD) were analyzed. Each quality control sample was prepared similarly to the samples. The method detection limit (MDL) for water was 0.006 μg L^{-1}, and the limit of quantitation (LOQ) was 0.02 μg L^{-1}. Since saliva samples were diluted 10-fold, the MDL for saliva was 0.06 μg L^{-1} and the LOQ was 0.2 μg L^{-1}. The MDL was calculated as 3 times the LRB value, and the LOQ was calculated as 10 times the LRB value.

Mean recovery of labeled internal standard spiked into water and saliva samples was 99% and 51%, respectively. The low recoveries of perchlorate spiked into saliva samples were due to ionization-suppression contributed by large biomolecules present in the samples. However, the reported concentrations in samples were corrected for internal standard recoveries. Additional purification of saliva by passage through Vivaspin 2 centrifugal filtration devices (CFDs; Sartorius Stedim Biotech, Goettingen, Germany) and centrifugation for 40 min at 2100*g* has been recommended to limit ionization-suppression (Oldi and Kannan, 2009).

Precision and accuracy of the analysis, reported as recoveries and relative standard deviation or relative percentage difference, are shown in Table 1. Perchlorate at known concentrations (0.1, 1 and 2 ng) was spiked into sample matrix as the LFDs, and passed through the entire analytical procedure. The LFBs had recoveries of 100 ± 12%, further confirming accuracy of the analysis. The CCC standards injected after every 10 samples showed deviations within 10% of the actual concentration values. Calibration standards injected with all samples generally produced curves with correlation coefficients >0.999, and the day-to-day slope variation was <1.3%. All results above the LOQ had ^{35}Cl:^{37}Cl ratios within the acceptable range of 3.12 ± 25%. Samples with concentrations below the LOQ were not included in the calculation of mean and median.

Table 1
Quality assurance and quality control parameters for the analysis of perchlorate in water and saliva.

QC sample type (concentration μg L^{-1})	*N*	Mean (μg L^{-1})	SD	RSD (%)	Average recovery (%)
Water					
Lab duplicate	6			4[a]	
Lab fortified duplicate	5				71
Lab fortified blank (2)	4	1.77	0.32	18	88
Low CCC (0.1)	1	0.10			100
Low CCC (0.5)	1	0.47			94
Mid CCC (1)	2	0.912	0.016	2	91
Mid CCC (5)	3	4.70	0.17	4	94
High CCC (20)	1	19.7			99
Saliva					
Lab fortified duplicate	5				94
Lab fortified blank (1)	5	0.915	0.034	4	92
Low CCC (0.1)	1	0.093			93
Low CCC (0.5)	4	0.45	0.20	45	90
Mid CCC (1)	1	0.99			99
Mid CCC (5)	1	4.6			93

SD = standard deviation, RSD = relative standard deviation, RPD = relative percentage difference, CCC = continuing calibration check.
[a] This value is RPD.

3. Results and discussion

3.1. Water

Concentrations of perchlorate in water samples from India are presented in Table 2. Perchlorate was detected, in most (76%) of the water samples analyzed, at concentrations above the LOQ of 0.02 μg L^{-1}; concentrations ranged from <0.02 to 6.9 μg L^{-1} (mean:

Table 2
Perchlorate concentrations in water samples from India as stratified by state and city.

State/city	Perchlorate concentration (μg L^{-1})				
	N	Min	Mean	SD	Max
Tamil Nadu					
Mettupalayam	13	<0.02	0.15	0.19	0.67
Coimbatore	13	<0.02	1.5	2.4	6.9
Ootacamund (Ooty)	2	0.070	0.092	0.031	0.113
Thoppur	1		0.25		
Krishnagiri	2	0.600	0.624	0.035	0.649
Bhavani	1		0.40		
Chennai	7	<0.02	0.22	0.21	0.37
Coonoor	1		0.063		
West Bengal					
Kolkata	4	<0.02	0.027		0.027
Bihar					
Patna	5	<0.02	0.134	0.072	0.221
Maharashtra					
Mumbai	4	0.044	0.057	0.020	0.088
Pondicherry					
Puducherry	5	<0.02	0.39	0.23	0.74
Karnataka					
Bengalaru	8	0.03	0.15	0.18	0.42
Overall	66	<0.02	0.42	1.08	6.9

0.42 ± 1.1 μg L^{-1}; median: 0.07 μg L^{-1}). Three water samples contained perchlorate concentrations above 1 μg L^{-1}; all these were groundwater samples from Coimbatore (Fig. 1). These three samples had been collected from wash basins from three different restaurants, where groundwater is used for washing purposes. However, drinking water served in the restaurants and in public facilities such as railway stations and airports contained perchlorate concentrations <0.05 μg L^{-1}. Coimbatore is a major industrial center and is known for its textile factories. It is plausible that industrial activities in Coimbatore contributed to groundwater contamination by perchlorate. Most restaurants and public facilities in India employ a secondary water treatment (of municipal water that had already undergone primary treatment in public treatment facilities) at the point of use, prior to serving to customers. The secondary treatment/purification of potable water, which usually involves passage through ion exchange resins, at the point of use, appears to effectively remove perchlorate. Perchlorate concentrations in drinking water and groundwater from other metropolitan cities such as Kolkata, Chennai and Bengaluru were <0.5 μg L^{-1}.

Water samples collected in this study were categorized as drinking water, groundwater, bottled water, surface water or rain water, to enable us to examine the differences in perchlorate concentrations based on the source and use. Mean concentrations of perchlorate in drinking water, groundwater, bottled water, surface water and rain water were 0.1, 1.0, <0.02, 0.05 and <0.02 μg L^{-1}, respectively (Fig. 2). High mean perchlorate concentrations in groundwater resulted from the contribution from three samples collected from Coimbatore, all of which had >1 μg perchlorate L^{-1}. After the exclusion of these three samples, the concentrations of perchlorate in groundwater (from cities such as Patna, Mettupalayam, Bengaluru and Chennai) were in the range of 0.02–0.74 μg L^{-1} (mean: 0.28 μg L^{-1}).

Very few studies have reported the occurrence of perchlorate in water from countries other than the United States. In a watershed contaminated with perchlorate from industrial sources in Japan, 19 out of 27 tap water samples had perchlorate concentrations exceeding 1 μg L^{-1}, with 13 of those containing more than 10 μg L^{-1} (Kosaka et al., 2007). Perchlorate concentrations in river waters, sampled from the middle and lower Tone River Basin, were generally 10–20 μg L^{-1} (Kosaka et al., 2007). The maximum concentrations of perchlorate in waters from the upper Tone River and Usui River were 340 and 2300 μg L^{-1}, respectively (Kosaka et al., 2007). Tap water sampled in Korea contained perchlorate concentrations ranging from 0.15 to 35 μg L^{-1} (Quinones et al., 2007), with a mean concentration of 10.4 μg L^{-1}. Water samples from the Nakdong and Yeongsan Rivers in that country also contained considerable levels of perchlorate (<0.05–60 μg L^{-1}) (Quinones et al., 2007). Analysis of perchlorate in 3865 public water supplies in the United States between 2001 and 2005 yielded concentration, for those samples with positive detections, of 9.85 μg L^{-1} (USEPA, 2008). Concentrations of perchlorate in drinking water and surface water samples from India were one to two orders of magnitude lower than the concentrations reported for Japan, Korea and the United States.

We did not detect perchlorate (at >0.02 μg L^{-1}) in any of the five sampled brands of bottled water from India. Concentrations of perchlorate in bottled water from China ranged from <0.002 to 2.01 μg L^{-1} (mean: 0.16 μg L^{-1}) (Shi et al., 2007). Perchlorate was found in 10 of the 21 bottled water samples collected from the United States at concentrations ranging from 0.07 to 0.74 μg L^{-1} (mean: 0.14 μg L^{-1}), while the remaining 11 samples had perchlorate concentrations below 0.05 μg L^{-1} (Snyder et al., 2005).

A groundwater survey in the United States reported that 45% of 326 samples contained less than 0.040 μg perchlorate L^{-1}; of the remaining samples, 13% were between 0.04 and 0.12 μg L^{-1}, and 42% were above 0.12 μg L^{-1} (Parker et al., 2008). Our groundwater samples from India showed lower concentrations of perchlorate than those reported in the United States. Relatively high concentrations of perchlorate in three groundwater samples from Coimbatore (above 1 μg L^{-1}), suggest the need for further investigation, to evaluate the sources of contamination. Perchlorate was not found in rain water samples from India. Mean concentrations of perchlorate in rain water from the United States ranged from <0.005 to 0.102 μg L^{-1} (overall mean: 0.014 μg L^{-1}) (Rajagopalan et al., 2009). The mean concentration reported for the US rain water samples was lower than LOQ in our present study.

3.2. Saliva

Perchlorate was found in saliva samples of Indian adults at concentrations above 0.2 μg L^{-1} (Table 3). Perchlorate concentrations as high as 4.7 μg L^{-1} were found in saliva, with an average value of 1.3 ± 1.3 μg L^{-1} (median: 0.91 μg L^{-1}). Fourteen of the 74 saliva samples had concentrations above 1 μg L^{-1}. When saliva samples were stratified by city, gender or age, no notable differences in perchlorate concentrations were found among the groups.

A recent study reported perchlorate concentrations in saliva samples collected from subjects in Albany, New York (Oldi and Kannan, 2009). The mean concentration of perchlorate in the saliva samples from Albany was 5.3 μg L^{-1}, and the maximum value was 37 μg L^{-1}. The mean concentration of perchlorate in saliva samples

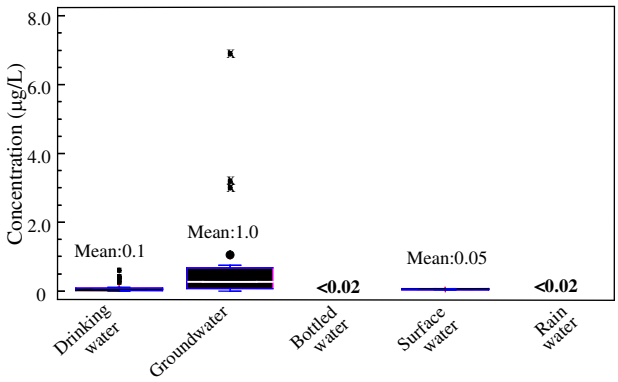

Fig. 2. Box and whisker plot of perchlorate concentrations in water samples from India (solid circle above the box, for groundwater, is median and symbols above the circle are outliers).

Table 3
Perchlorate concentrations in saliva from India as stratified by city, gender and age.

	N	Perchlorate concentration (μg L^{-1})			
		Min	Mean	SD	Max
City					
Mettupalayam	19	<0.2	0.60	0.52	1.67
Chennai	8	<0.2	1.34	0.86	2.50
Patna	25	<0.2	0.70	0.63	2.05
Bengalaru	22	<0.2	2.7	1.7	4.7
Gender					
Male	52	<0.2	1.3	1.2	4.7
Female	22	<0.2	1.3	1.5	4.7
Age (years)					
18–24	9	<0.2	0.25	0.17	0.48
25–34	16	<0.2	1.3	1.1	3.4
35–44	22	<0.2	1.6	1.6	4.7
45–54	12	<0.2	1.10	0.61	2.05
55–64	10	<0.2	1.9	2	4.7
>65	5	<0.2	0.34	0.16	0.49
Overall	74	<0.2	1.3	1.3	4.7

from the Indian donors was approximately fivefold lower than the concentrations reported for Albany.

In summary, perchlorate was detected in both drinking water and saliva samples collected in India. Concentrations of perchlorate measured in drinking water in India are one to two orders of magnitude lower than the concentrations reported for more industrialized countries (USA, Japan and Korea). Concentrations of perchlorate in water samples did not exceed the US EPA's interim health advisory level, 15 $\mu g\,L^{-1}$, for perchlorate in drinking water. Based on the mean concentration of 0.1 $\mu g\,L^{-1}$ in drinking water from India, exposure of perchlorate for a 70-kg adult drinking 2L d^{-1} of water would be 0.003 $\mu g\,kg^{-1}$ bw d^{-1}, which is <1% of the reference dose established by the EPA. However, concentrations in saliva exceeded the concentrations in the water samples, with several saliva samples containing concentrations above 1 $\mu g\,L^{-1}$, suggesting the presence of other sources of perchlorate exposure for the Indian population. Studies have reported foodstuffs to be a source of perchlorate in the United States (Sanchez et al., 2005; Murray et al., 2008), and further investigation is needed to examine the sources of exposure of the Indian population to perchlorate.

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