Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 68 (2006) 1457–1462
Talanta
www.elsevier.com/locate/talanta

A cleanup method for perchlorate determination in urine

Qiuqiong Cheng, Fujun Liu, Jaclyn E. Cañas, Todd A. Anderson *

The Institute of Environmental and Human Health, Department of Environmental Toxicology, Texas Tech University, Box 41163, Lubbock, TX 79409-1163, USA

Received 9 May 2005; received in revised form 27 July 2005; accepted 27 July 2005
Available online 31 August 2005

Abstract

There is increasing concern about perchlorate exposure because of perchlorate's potential effects on organisms as a thyroid hormone disruptor, as well as its contamination of the environment being much more widespread than previously thought. Perchlorate is excreted primarily into urine, therefore, evaluating perchlorate residues in urine should be a reasonable approach for determining exposure and if successful could be used as an effective biomarker of perchlorate exposure. Since the presence of ions and other biomolecules in matrices like urine usually confounds accurate determination of perchlorate by ion chromatography, it is necessary to develop efficient methods for perchlorate determination in these matrices. We developed a method that reduces the background signal of urine, which is typically the problem with the analysis of biological fluids and tissues by ion chromatography. Relatively high recovery of perchlorate was shown. In cow urine samples spiked with perchlorate at 2.5, 10, and 100 μg/L, perchlorate recoveries were 67% ± 2.5, 77% ± 3.6, and 81% ± 1.7 (mean ± S.D.), respectively. In addition, the detection limit was as low as 12.6, 12.3, and 18.7 μg/L in cow, vole, and human urine samples, respectively.

Keywords: Perchlorate; Cleanup method; Urine; Ion chromatography

1. Introduction

Perchlorate is an environmental contaminant that can become a problem in animals at certain exposure levels, targeting mainly the thyroid gland. It is well documented that perchlorate competitively inhibits iodide uptake by the thyroid gland [1–3], interfering with normal thyroid function and resulting in reduced production of thyroid hormones (T_4 and T_3) and increased production of thyroid-stimulating hormone (TSH) [4–6]. Thyroid hormones play key roles in development, growth, and metabolism in animals [4]. Therefore, exposure to perchlorate may eventually interfere with normal growth and could arrest or delay brain development, especially in the fetus or infant.

In the United States, discovery of perchlorate contamination in the environment continues to increase, particularly in water systems. According to documents, perchlorate was manufactured or utilized in 49 states, among which contamination occurs in 30 states [7]. Because perchlorate salts are extremely water soluble and kinetically stable, the perchlorate anion is exceedingly mobile in aqueous systems and can persist for many years under normal ground and surface water conditions. Perchlorate can be taken up by plants, animals, and humans directly or indirectly such as through the food chain. Uptake of perchlorate into vegetables, such as lettuce, has been reported [8–11,29]. Perchlorate has also been detected in tissues of aquatic plants and animals in the vicinity of contaminated sites [12–15]. Concerns on perchlorate contamination have increased recently after perchlorate was found in supermarket milk, human milk, and other food items in the U.S. [16–18], suggesting that perchlorate exposure potential to animals and humans might be much higher than previously thought.

Urine was found to be the primary excretion route for perchlorate in animals [1,19–22], therefore, monitoring of perchlorate content in urine could be a useful biomarker of perchlorate exposure provided the urine is collected shortly after perchlorate exposure (<24 h). Although high levels of perchlorate contamination (≥1 μg/mL perchlorate in ground-

* Corresponding author. Tel.: +1 806 885 4567; fax: +1 806 885 4577.
E-mail address: todd.anderson@ttu.edu (T.A. Anderson).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.07.052

water) have been reported, low or trace perchlorate contamination (≤20 μg/L) is more commonly the case [30]. Biomarkers of perchlorate effects, such as changes in thyroid hormone profiles and thyroid histopathology may not be as efficient as a biomarker of exposure (i.e., urine perchlorate content) because the aforementioned biomarkers of effect are manifested only at relatively high perchlorate exposure. Therefore, at environmentally relevant perchlorate contamination, urine analysis for perchlorate may be a good choice for environmental monitoring. However, accurate quantitative determination of perchlorate in biological fluids is often problematic because biological fluids can contain additional ions, proteins, lipids, sugars, and other biomolecules which may confound accurate determination of contaminants of interest. Previous studies have reported that proper sample preparation can minimize interferences and reduce background conductivity for ion chromatography determination of perchlorate in plant tissues, animal tissues, and blood plasma [23–25], but to our knowledge, there are few efficient methods for perchlorate determination in urine samples. Since perchlorate is primarily excreted and eliminated through urine, a well-developed preparation method for urine sample analysis for perchlorate would contribute greatly to more accurately evaluating exposure of animals or humans to perchlorate.

The purpose of the current study was to develop a cleanup method for urine samples to eliminate interferences for perchlorate determination using ion chromatography. Our goal was to reduce background conductivity of urine components while maintaining a low detection limit for perchlorate in urine.

2. Materials and methods

2.1. Chemicals

A perchlorate (ClO_4^-) standard solution was obtained as a custom standard from AccuStandard, Inc. (New Haven, CT). Sodium hydroxide (50%, w/w) aqueous solution was purchased from Fisher Scientific (Fair Lawn, NJ). Acetonitrile (HPLC grade) was purchased from EMD (Gibbstown, NJ). All solutions were prepared in 18.2 MΩ Milli-Q water.

2.2. Sample source and treatment

The urine samples used in the current study were from voles (*Microtus ochrogaster*), cattle, and human (female). The cow urine samples were provided by the Department of Animal and Food Science, Texas Tech University (Lubbock, TX). Vole urine samples were obtained from a breeding colony housed at Texas Tech University. Human urine samples were obtained from volunteers in Lubbock, TX. Using IC analysis and LC/MS confirmation, perchlorate was not detected in the cattle and human urine samples, however, trace perchlorate was detected in the vole urine.

In order to determine the efficiency of different cleanup methods, a perchlorate standard solution was spiked into cattle urine samples; the final concentration was 100 μg/L. The samples were analyzed for perchlorate using ion chromatography after preparation by different cleanup methods, and the efficiency and recovery of perchlorate in the samples was determined. Based on this preliminary result, the most efficient method was chosen for further evaluation of its applicability to different urine sources (vole and human urine) with spiked perchlorate (2.5, 10, and 100 μg/L). In addition, urine samples collected from cows inhabiting a perchlorate-contaminated site were used to evaluate the applicability of the cleanup method for field samples.

2.3. Sample preparation procedure

Solid phase extraction (SPE) cartridges were used in the cleanup process of urine samples for ion chromatography analysis. Ten types of SPE cartridges were evaluated, individually or in combination, to determine cleanup efficiency. The SPE cartridges tested included quaternary amine (CUQAX 100 mg), quaternary amine with hydroxide (CHQAX 100 mg), quaternary amine-acetate (CAQAX 100 mg), strong anion exchange (Strata SAX 100 mg), N-2 aminoethyl (PSA 500 mg) combined with alumina-neutral (Al-N 1 g), octadecyl (C18 1 g) combined with PSA (500 mg), hydrophobic and aminopropyl (NAX 1 g), NAX combined with Al-N, NAX combined with PSA, and C18 combined with Al-N. CUQAX, CHQAX, CAQAX, PSA, and NAX were obtained from United Chemical Technologies, Inc. (Bristol, PA). Strata SAX was purchased from Phenomenex (Torrance, CA). C18 cartridges were purchased from Honeywell B&J (Muskegon, MI), and Al-N was purchased from J.T. Baker (Phillipsburg, NJ).

Depending on the sorbent, SPE cartridges were conditioned as appropriate prior to use. For CUQAX, CHQAX, and CAQAX, 0.4 mL of urine sample was loaded, 1 mL Milli-Q water (>18 MΩ) was used to elute the sample through the cartridge, and the eluate was diluted to a final volume of 2 mL with Milli-Q water. For SAX, 0.5 mL of urine sample was loaded, then 2.5 mL NaOH (20 mM in 15% acetonitrile solution) was added to elute the sample through the cartridge following consecutive washing with 1 mL Milli-Q water and 1.5 mL NaOH (20 mM in 15% acetonitrile solution). For NAX, following the loading of 0.8 mL urine sample and washing with 0.6 mL DI water, 4 mL Milli-Q water was used to elute the cartridge. For the combination cartridges with NAX and Al-N or NAX and PSA, the 4 mL Milli-Q water eluted from NAX was further processed through Al-N or PSA. For the case of C18 in combination with PSA or Al-N, 0.8 mL urine sample was loaded onto the cartridge, followed by 4 mL Milli-Q water to elute the cartridge. The eluate then was loaded and eluted through PSA or Al-N cartridges. In the case of PSA combined with Al-N, 0.8 mL sample was first diluted to 4 mL with Milli-Q water and then eluted through PSA cartridge, followed by Al-N cartridge. All final eluates

were filtered and analyzed by ion chromatography without further dilution.

2.4. Sample analysis

A method similar to EPA Method 314.0 [26] was followed to determine perchlorate in all samples. The analysis was performed on a Dionex DX-500 Ion Chromatography System equipped with a GP50 gradient pump, a CD20 conductivity detector, and an AS40 automated sampler (Dionex Corp.). PeakNet® chromatography software was used to control the system. Ion separation was conducted with a Dionex IonPac AS16 (250 mm × 4.0 mm i.d.) analytical column after a Dionex guard column (AG16). Conditions for the system were as follows: flow rate = 1.0 mL/min; eluent = 50 mM sodium hydroxide; injection volume = 1000 μL. Ion detection was by suppressed conductivity in the external water mode. Computer-generated peak areas were used to measure sample concentrations in an external standard mode.

3. Results and discussion

3.1. Efficiency of various cleanup procedures

Among the tested SPE cartridges as individuals or in combination, NAX and its combination with other cartridges reduced the background conductivity and interference dramatically (Fig. 1). For a 5X-diluted urine sample, cleanup by NAX in combination with PSA or Al-N cartridges resulted in very low background conductivity and much less interference compared with other cleanup procedures. As illustrated in Fig. 1, cleanup with NAX combined with Al-N gave the best result in terms of reducing interference and background signal. Furthermore, cleanup by NAX, NAX plus PSA, and NAX plus Al-N cartridges showed much higher perchlorate recoveries among all tested cleanup procedures

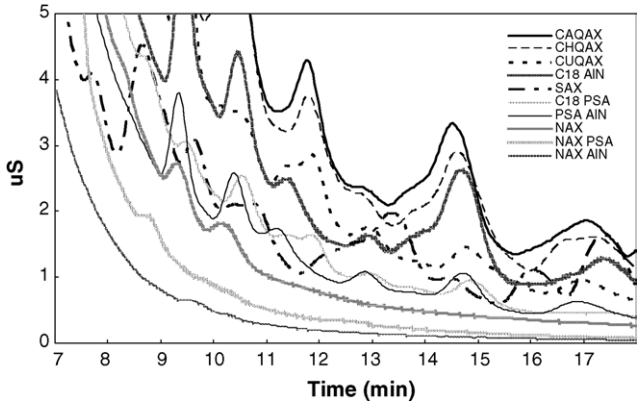

Fig. 1. Ion chromatograms of cow urine samples (no perchlorate) after being processed via various cleanup procedures. Different cartridges or combinations of cartridges were tested for cleanup efficiency in terms of reduced background signal and interferences. Cartridge labels in the legend are listed in the same descending order as the overlaid chromatograms.

Table 1
Recoveries of perchlorate in perchlorate-spiked cow urine (100 μg/L) cleaned using various solid phase extraction (SPE) cartridges

Phase	% Recovery (mean ± S.D.)	Phase	% Recovery (mean ± S.D.)
NAX	74 ± 7.5	PSA Al-N	70 ± 1.9
NAX Al-N	70 ± 6.0	CUQAX	29 ± 2.8
NAX PSA	73 ± 3.9	CHQAX	25 ± 5.5
C18 Al-N	75 ± 1.1	CAQAX	24 ± 3.6
C18 PSA	43 ± 3.4	SAX	30 ± 11.7

(Table 1). The sorbent in NAX cartridges is composed of a silica backbone with an anion exchanger (aminopropyl) and a hydrophobic carbon chain (C8). When a urine sample, which contains abundant positively charged ions, is applied to the NAX cartridge, the positively charged ions are not retained by the anion exchanger (the amine groups) and are eluted, whereas the negatively charged ions can interact with the amine groups. Hydrophobic molecules in the urine sample, such as organic-based compounds (i.e., carbohydrates, proteins, etc.), bind to the C8 phase. Therefore, the negatively charged and hydrophobic compounds can be held in the cartridge after urine sample application. Because the amine groups are weak anion exchangers, perchlorate anions do not strongly interact with them. Thus, perchlorate anions can be eluted by water following the urine sample application, while hydrophobic compounds and other weak anions are left in the cartridge through interaction with C8 groups and amine groups, respectively. However, cleanup with the NAX cartridge alone gave a relatively high background signal due to the high total conductivity. The combination of NAX with PSA and particularly with Al-N showed significant improvement (Fig. 1). Work in our lab has shown that Al-N was effective in reducing background and interference in a variety of other matrices (unpublished data). Here, we also found this type of cartridge works efficiently for urine samples as well when combined with NAX. Although the combination of C18 and PSA has a similar functional composition as NAX, this combination did not show similar results as NAX in terms of background signal and perchlorate recovery.

CAQAX, CHQAX, CUQAX (i.e., QAXs), and SAX cartridges contain quaternary amine anion exchangers. In the current study, the way we used them as cleanup cartridges for perchlorate determination is based on a preelution principle: loading the sample on the cartridge, washing the cartridge with water or other solution, and then eluting perchlorate from the cartridge with a proper solution. It turned out that these cartridges were not as effective as either NAX alone or in combination with Al-N or PSA in reducing background signal and interferences (Fig. 1). In addition, they gave poor perchlorate recovery (Table 1). The QAX-type cartridges do not bind perchlorate strongly, and thus perchlorate may be eluted by the washing solution. Therefore, we skipped the washing step, and directly eluted perchlorate with water without washing after loading the sample on the cartridges. However, there were co-eluting ions with perchlorate in the eluate

and high background signal during ion chromatography analysis. Compared to QAX, SAX is a much stronger anion exchanger. However, the anion exchange capacity appears to vary depending on the vendor. In the current study, we found that perchlorate can be eluted from SAX cartridges from Phenomenex (Torrance, CA), but not from another provider/brand by using 20 mM (or higher) NaOH as an eluent. Because of the relatively small mass of the sorbent bed in the cartridge (100 mg), it was not easy to separate perchlorate from other interferences. As a result, high background conductivity and interferents were observed. It was very difficult to elute perchlorate if larger SAX cartridges with more sorbent bed were used. Furthermore, perchlorate recovery was low using SAX cartridges. SAX is a strong anion exchanger, which holds perchlorate tightly, causing difficulty in eluting perchlorate using water. Even with 20 mM or higher NaOH (a stronger eluent), perchlorate could not be eluted with a limited amount of eluent and therefore, low recovery of perchlorate was observed. In addition, poor reproducibility was observed using this cleanup method. This may be caused by inconsistent elution rates. Therefore, controlling the flow rate of eluent at a constant value would probably improve the reproducibility of the SAX cartridge in cleanup of urine samples.

The combinations of C18 with Al-N and PSA with Al-N produced high perchlorate recoveries similar to NAX in combination with PSA or Al-N (Table 1), but they were less effective in reducing background conductivity and interferences. Cleanup efficiency for these four types of cleanup procedures for both blank and spiked urine samples are presented in Fig. 2. For the combination of either C18 with Al-N or PSA with Al-N, the perchlorate peak (peak A in Fig. 2a) was very close to an interference peak (peak B in Fig. 2b), leading to the appearance of a shoulder-peak near the retention time for perchlorate (retention times less than 0.5 min difference). Consequently, it would be easy to mistakenly regard the interference peak as perchlorate in samples which contain no perchlorate. In contrast, no interference peak was observed in samples processed by a combination of NAX with Al-N or with PSA. The cleanup of urine samples for perchlorate determination using NAX combined with Al-N or PSA produced high recovery, low background conductivity, and no adjacent interfering peak.

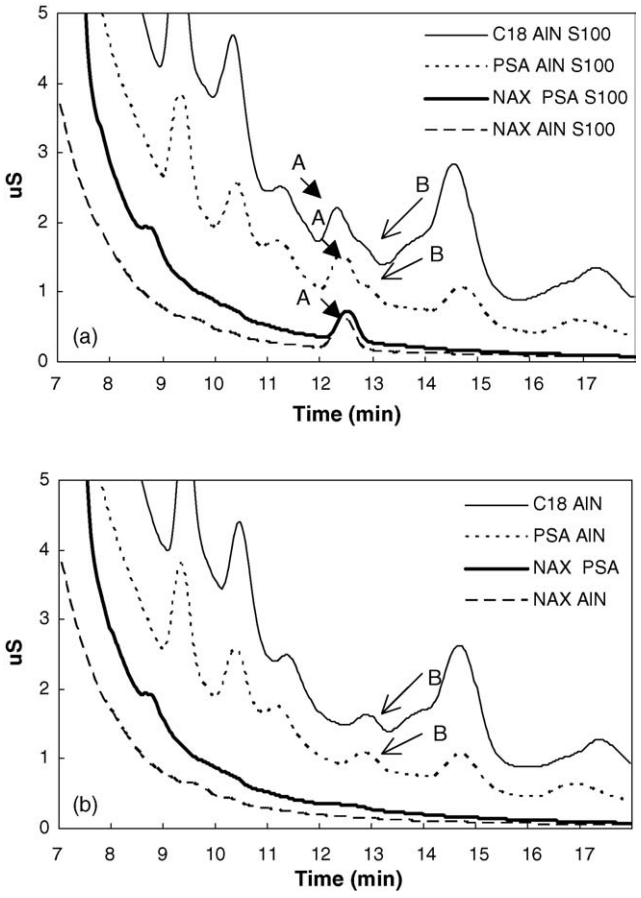

Fig. 2. Ion chromatograms showing different efficiencies of four cleanup procedures (NAX combined with Al-N, NAX with PSA, C18 with Al-N, and PSA with Al-N) for perchlorate determination in urine samples spiked with perchlorate (spike concentration was 100 μg/L) (a) and without perchlorate (b). Peak A in (a) is perchlorate. There is a shoulder peak adjacent to peak A with combination of C18 and Al-N or PSA and Al-N as cleanup procedure. The adjacent peak B is an interference peak shown in (b). Cartridge labels in the legend are listed in the same descending order as the overlaid chromatograms.

3.2. Accuracy and precision of NAX plus Al-N for cleanup of different types of urine matrices

Since NAX combined with Al-N proved to be efficient for cleanup of cow urine samples for perchlorate determination, we further evaluated the accuracy and precision of this cleanup procedure for different types of urine samples spiked with perchlorate. Results are presented in Table 2. Perchlorate recoveries (±S.D.) were 67% ± 2.5, 77% ± 3.6, and 81% ± 1.7 for cow urine samples spiked with perchlorate at 2.5, 10, and 100 μg/L, respectively. The highest perchlorate recovery was found for vole urine samples, with 97% ± 4.1, 83% ± 5.1, and 86% ± 1.6 in the 2.5, 10, and 100 μg/L perchlorate samples, respectively. Recovery was lowest in human urine, with 76% ± 0.8, 46% ± 1.0, and 56% ± 3.4, respectively. Since Al-N (without conditioning) does not adsorb perchlorate, the reduced perchlorate recovery in human urine samples is probably caused by the NAX cartridge. We tested the effect of different

Table 2
Accuracy and precision for NAX combined with Al-N as cleanup cartridges for perchlorate determination in different types of urine using ion chromatography

Perchlorate spike (μg/L)	% Recovery (mean ± S.D.)		
	Cow	Vole	Human
2.5	67 ± 2. 5	97 ± 4. 1	76 ± 0.8
10	77 ± 3.6	83 ± 5.1	46 ± 1.0
100	81 ± 1.7	86 ± 1.6	56 ± 3.4

Table 3
Physical properties (pH and conductivity) of the different types of urine samples used in experiments

Property	Cow	Vole	Human
pH	9.05	8.37	7.10
Conductivity (mS)	15.39	19.29	23.04

elution volumes of Milli-Q water on perchlorate recovery, and found that perchlorate was eluted primarily within the first 4 mL of water, with 92 and 63% recovery of spiked perchlorate (100 μg/L) in cow urine and in human urine, respectively.

The pH profile of different urine samples (Table 3) may cause differential perchlorate recovery. The pH of human and cow urine was 7.10 and 9.05, respectively. The pH may alter perchlorate elution from NAX by affecting the amine groups. Perchlorate recovery was increased dramatically if 20 mM NaOH was used as eluent, but with interferences. In addition, conductivity of urine samples (Table 3) may also have an influence on cleanup using NAX. Human urine (with higher conductivity) may have additional ions which could compete with perchlorate's interaction with the amine groups.

Considering the recoveries and reduction of background signal, the limit of detection (LOD) for perchlorate in cow, vole, and human urine samples was 12.6, 12.3, 18.7 μg/L, respectively (based on $S/N = 3$).

3.3. Application of cleanup method (NAX plus Al-N) to urine samples from a field study

It is well known that perchlorate is excreted primarily via urine, therefore, monitoring perchlorate residues in urine should be a sensitive biomarker for perchlorate exposure. The current study provided a promising cleanup method for perchlorate determination using ion chromatography in a variety of urine types, including cattle, voles, and human urine. In addition, the application of this cleanup method provides for the detection of perchlorate in urine as low as 12.6 μg/L. To our knowledge, this is the lowest reported detection limit of perchlorate in urine or similar biological matrices using conventional ion chromatography.

We also attempted to use this cleanup method to evaluate perchlorate in urine samples from the field. The urine samples ($n = 4$) were from cattle inhabiting two different pastures where perchlorate was detected in certain drinking water samples (ponds) as high as 100 μg/L. Cattle on these pastures were not restricted to water supplies containing perchlorate; most of the ponds did not contain perchlorate and there was some anecdotal evidence that the cattle were avoiding the perchlorate-contaminated water. Using the cleanup method described, we did not detect perchlorate in any of the urine samples, which was consistent with analysis results of the corresponding plasma samples in which no perchlorate was detected (unpublished data). Confirmation analysis of all samples from the contaminated site using LC/MS revealed that one urine sample contained perchlorate (concentration = 3.45 μg/L after 8× dilution). This particular urine sample was very dirty with many interferences and high background conductivity even after cleanup: thus perchlorate was not detected by conventional IC.

Compared to the cow urine samples provided by the Department of Animal and Food Science, Texas Tech University, used in the laboratory portion of this study, the urine samples collected from the field were much darker in color. The average pH of these cow urine samples was 7.86 ± 0.11 (±S.D.), and the conductivity ranged from 11.74 to 68.68 mS. These field urine samples had much higher background and poor recovery if 0.8 mL sample was loaded onto the NAX and eluted with 4 mL Milli-Q water. These more complex urine samples appeared to exceed the capacity of NAX, resulting in only partial interaction of sample with the NAX. Therefore, we had to revise the procedure slightly (only 0.4 mL or less sample was loaded onto NAX and eluted with 4 mL Milli-Q water or 5 mM NaOH after washing with 0.6 mL Milli-Q water). Nonetheless, perchlorate was not detected in these animal urine samples by ion chromatography.

To monitor perchlorate exposure in wild animals or humans, two categories of endpoints are widely used: biomarkers of exposure and biomarkers of effect. Perchlorate determination in blood or tissue matrices are examples of the former [12,13,25]; the latter includes iodide uptake, thyroid hormone status/profile, and thyroid histopathology [2,3]. However, it appears that these biomarkers are useful primarily in cases of high perchlorate contamination/exposure. At environmentally relevant exposures, these biomarkers are not as effective [25,27,28]. For biomarkers of exposure, urine analysis may be more sensitive than blood residue analysis since urine is a primary excretion pathway for perchlorate elimination by animals. Therefore, an efficient cleanup method for perchlorate in urine is useful and should contribute to environmental monitoring of perchlorate contamination.

Acknowledgments

The authors thank Joanna Johnson and Dr. Leon Glass from United Chemical Technologies, Inc. (Bristol, PA) for providing SPE cartridge samples. The authors also thank Cassie Abney and Dr. Michael Galyean from the Department of Animal and Food Science, Texas Tech University, for cow urine samples. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the 311 HSW/AFIERA or the U.S. Government. This research was supported in part by the U.S. Department of Defense contract CU1141, through the Strategic Environmental Research and Development Program (SERDP) under a Cooperative Agreement IERA-99-001 with the USAF, Institute for Environment, Safety, and Occupational Health, Brooks AFB, TX.

References

[1] K.O. Yu, L. Narayanan, D.R. Mattie, R.J. Godfrey, P.N. Todd, T.R. Sterner, D.A. Mahle, M.H. Lumpkin, J.W. Fisher, Toxicol. Appl. Pharmacol. 182 (2002) 148–159.
[2] J.E. Lawrence, S.H. Lamm, L.F. Braverman, Thyroid 10 (2000) 659–663.
[3] M.A. Greer, G. Goodman, R.C. Pleus, S.E. Greer, Environ. Health Perspect. 110 (2002) 927–937.
[4] J. Wolff, Pharmacol. Rev. 50 (1998) 89–105.
[5] O.P. Soldin, L.E. Braverman, S.H. Lamm, Therapeut. Drug Monitor. 23 (2000) 316–331.
[6] National Research Council, Health Implications of Perchlorate Ingestion, The National Academies Press, Washington, DC, 2005.
[7] J. Lee, http://www.mindfully.org/Water/2004/Chemical-Water-Too-Much2mar04.htm.
[8] S. Susarla, N. Wolfe, S. McCutcheon, Preprints of Extended Abstracts, Division of Environmental Chemistry, 218th ACS National Meeting, ACS, vol. 39, No. 2, New Orleans, LA, August 22–26, 1999, pp. 66–68.
[9] C. Hogue, Chem. Eng. News 81 (2003) 11.
[10] L. Yu, J.E. Cañas, G.P. Cobb, W.A. Jackson, T.A. Anderson, Ecotoxicol. Environ. Safety 58 (2004) 44–49.
[11] W.A. Jackson, P. Joseph, P. Laxman, K. Tan, P.N. Smith, L. Yu, T.A. Anderson, J. Agric. Food Chem. 53 (2005) 369–373.
[12] P.N. Smith, C.W. Theodorakis, T.A. Anderson, R.J. Kendall, Ecotoxicology 10 (2001) 305–313.
[13] P.N. Smith, L. Yu, S.T. McMurry, T.A. Anderson, Environ. Pollut. 132 (2004) 121–127.
[14] K. Tan, W.A. Jackson, T.A. Anderson, J.H. Pardue, Water Res. 38 (2004) 4173–4185.
[15] K. Tan, T.A. Anderson, M.W. Jones, P.N. Smith, W.A. Jackson, J. Environ. Qual. 33 (2004) 1638–1646.
[16] A.B. Kirk, E.E. Smith, K. Tian, T.A. Anderson, P.K. Dasgupta, Environ. Sci. Technol. 37 (2003) 4979–4981.
[17] A.B. Kirk, P.K. Martinelango, K. Tian, A. Dutta, E. Smith, P.K. Dasgupta, Environ. Sci. Technol. 39 (2005) 2011–2017.
[18] A.J. Krynitsky, R.A. Niemann, D.A. Nortrup, Anal. Chem. 72 (2004) 5518–5522.
[19] M. Anbar, S. Guttmann, Z. Lewitus, Int. J. Appl. Rad. Isotopes 7 (1959) 87–96.
[20] S.J. Goldman, J.B. Stanbury, Endocrinology 92 (1973) 1536–1538.
[21] H.G. Pena, W.V. Kessler, J.E. Christian, T.R. Cline, M.P. Plumlee, Poult. Sci. 55 (1976) 188–201.
[22] P. Batjoens, H.F. De Brabander, L. T'Kindt, Anal. Chim. Acta 275 (1993) 335–340.
[23] J.J. Ellington, J.J. Evans, J. Chromatogr. A 898 (2000) 193–199.
[24] T.A. Anderson, T.H. Wu, Bull. Environ. Contaminat. Toxicol. 68 (2002) 684–691.
[25] Q. Cheng, L. Perlmutter, P.N. Smith, S.T. McMurry, W.A. Jackson, T.A. Anderson, J. Agric. Food Chem. 52 (2004) 3456–3461.
[26] D.P. Hautman, D.J. Munch, A.D. Eaton, A.W. Haghani, EPA 815-B-99-003, U.S. Environmental Protection Agency, Cincinnati, OH, 1999.
[27] F.X. Li, D.M. Byrd, G.M. Deyhle, D.E. Sesser, M.R. Skeels, S.R. Katkowsky, S.H. Lamm, Teratology 62 (2000) 429–431.
[28] Z. Li, F.X. Li, D. Byrd, G.M. Deyhle, D.E. Sesser, M.R. Skeels, S.H. Lamm, J. Occup. Environ. Med. 42 (2000) 200–205.
[29] C.A. Sanchez, R.I. Krieger, N. Khandaker, R.C. Moore, K.C. Holts, L.L. Neidel, J. Agric. Food Chem. 53 (2005) 5479–5486.
[30] EPA, Known Perchlorate Releases in the U.S., March 2005. http://www.epa.gov/fedfac/pdf/detect0305.pdf.