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Perchlorate, Nitrate, Thiocyanate, and Iodide Levels in Chicken Feed, Water, and Eggs from Three Farms

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Perchlorate is an inhibitor of iodide uptake that is found widely in the environment. Given the potential for perchlorate accumulation during egg formation and the widespread consumption of eggs, it is important to examine eggs as a source of exposure to perchlorate and other potential inhibitors of iodide uptake (nitrate and thiocyanate). This study was conducted to determine potential human exposure to perchlorate from eggs produced by chicken flocks consuming differing amounts of perchlorate. The mean concentrations of perchlorate (7.16 ± 1.99 μg/kg of dry weight), nitrate (2820 ± 2100 μg/kg of dry weight), thiocyanate (574 ± 433 μg/kg of dry weight), and iodide (2980 ± 1490 μg/kg of dry weight) in eggs ($n = 180$) from 15 chicken houses on 3 U.S. farms were determined. Chickens secreted into eggs an average of 23% of the perchlorate ingested from feed and water. Perchlorate levels in eggs were positively correlated with perchlorate intake ($p < 0.001$). Increased intake of perchlorate, nitrate, and thiocyanate was associated with decreased iodide levels in eggs, possibly indicating a competitive transport mechanism, such as sodium−iodide symporter. It was estimated that egg consumption contributes minimal perchlorate (~0.040 μg) compared to the average total intake of ~10.5 μg for U.S. adults. Additionally, it was found that egg consumption was not associated with increased perchlorate exposure in 2820 individuals from the National Health and Nutrition Examination Survey (p value for the difference of least-squares means, pDiff = 0.225). From these findings it was concluded that, although chickens secrete perchlorate in eggs, eggs do not appear to be a significant source of perchlorate exposure for adults in the United States.

KEYWORDS: Perchlorate; nitrate; thiocyanate; iodide; eggs; egg formation

INTRODUCTION

Perchlorate is an inorganic anion that can disrupt thyroid function by competitively inhibiting iodide uptake at the sodium−iodide symporter (NIS). NIS-mediated iodide uptake can also be inhibited by thiocyanate and nitrate (*1*−*3*). Prolonged inhibition of iodide uptake can lead to decreased thyroid hormone production and ultimately could result in hypothyroidism. Recent data indicate that exposure to these NIS inhibitors is widespread, albeit at estimated doses below the reference dose (*4*). Subsequent regression analysis found that increased urinary perchlorate levels were associated with decreased thyroxine and increased thyroid-stimulating hormone in women with urinary iodine of <100 μg/L (*5*). Therefore, it is important to understand the relative levels of perchlorate, nitrate, thiocyanate, and iodide to which people are exposed through food versus other sources (e.g., water).

Perchlorate is a powerful oxidant that is used in rocket fuel, munitions, blasting operations, and fireworks (*6*). In addition, perchlorate can form naturally in the atmosphere and accumulate in arid regions (*7, 8*). Perchlorate is freely soluble in water and stable in aerobic solutions. Preliminary data indicate that perchlorate has been detected at least once in 4.1% of U.S. public drinking water systems, with levels ranging from the detection limit of 4 μg/L to a maximum of 420 μg/L (*9*). Recent studies indicate that perchlorate is also found in a variety of foods and food crops (*10*−*15*), the milk from cows (*11, 16*−*18*), and human breast milk (*17, 19*). Food is a significant source of perchlorate exposure in the United States (*11, 20*); efforts are ongoing to characterize which foods contribute most to perchlorate exposure. Milk and dairy products consistently contain perchlorate (*11*), likely because of active transport of perchlorate by the sodium−iodide symporter during milk formation (*21*). Active transport of perchlorate across cell membranes in other physiological systems has been confirmed in vitro (*22*), and it raises the possibility of active transport and concentration of perchlorate into chicken eggs during oogenesis.

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Eggs are a potential source of iodine intake, as well as perchlorate, thiocyanate, and nitrate exposure. There is evidence that perchlorate accumulates in crops that are typically used as poultry feed (*18, 23*). Pena et al. found significant perchlorate accumulation in developing and fully formed eggs produced by chickens following an intramuscular injection of radioactive perchlorate (*24*), raising concerns that chickens consuming perchlorate in feed or water may concentrate perchlorate in the edible portion of eggs. Previous studies have also shown that thiocyanate (*25*) and nitrate (*26*) are found in the edible portion of chicken eggs, and chickens consuming drinking water contaminated with nitrate produced eggs with elevated nitrate contents (*26*). Although these studies have been carried out for thiocyanate and nitrate, there have been no studies of the perchlorate levels in eggs produced by chickens consuming perchlorate in feed and water. Given the widespread consumption of eggs and evidence of perchlorate accumulation during egg formation, it is important to examine eggs as a potential source of exposure to perchlorate and other inhibitors of iodide uptake (nitrate and thiocyanate). This study was conducted to estimate human exposure to perchlorate, thiocyanate, and nitrate from eggs produced by chicken flocks consuming differing amounts of perchlorate in feed and water.

MATERIALS AND METHODS

Sampling. Three conventional egg farms were selected: one each from northern California, southern California, and Arizona. The farms were selected to have similar size (~1 million laying hens) and breed (White Leghorn) but differing in drinking water perchlorate levels. One of the farms used water from the lower Colorado River, which is known to contain perchlorate at low parts per billion levels (*15*). The selected farms implemented no changes in feed composition or water source during the week of the study. The three farms are not representative of conventional U.S. egg farms. Five chicken houses from differing locations on each of the three farms were selected for further sampling, resulting in egg samples collected from a total of 15 chicken houses. Houses 1−5 are from farm I, houses 6−10 from farm II, and houses 11−15 from farm III. From each chicken house we collected a sample of chicken feed, water, and 12 eggs. Additionally, we purchased seven free-range eggs that were produced in an area of California with no known perchlorate contamination.

Sample Preparation. The shell of each collected egg was discarded and the remaining edible portion individually homogenized and frozen. Each homogenized sample of egg or chicken feed was lyophilized overnight (Genesis, VirTis, Gardiner, NY) to remove moisture. The wet weight and the dry weight were recorded before and after the lyophilizing process, respectively. The lyophilized samples were stored at −20 °C until analysis. Approximately 1 g of dried aliquot was suspended in 15 mL of deionized water (>18 MΩ) and sonicated at 40 °C for 1 h. During the sonication process, the samples were mixed thoroughly by vortexing every 15 min. After the sonication process, the samples were placed in a refrigerator for 12−16 h. The samples were centrifuged at 3750 rpm for 30 min (Beckman Coulter, Allegra x12R, Fullerton, CA), and the supernatant was transferred to a new tube. The supernatant was filtered through a Spin-X centrifuge tube filter (0.22 μm nylon polypropylene tube, Corning Inc., NY) by centrifuging at 13000 rpm (Sorvall Pico, Thermo Fisher Scientific Inc., Waltham, MA) for 1 h. Subsequently, a 500 μL filtrate was mixed with 500 μL of internal standard mixture containing stable isotope-labeled internal standards ($^{18}O_4$-ClO_4, ^{15}N-NO_3, ^{15}N-SCN) plus 3-chlorobenzene (internal standard for iodide) and analyzed for perchlorate, nitrate, thiocyanate, and iodide.

Sample Analysis for Perchlorate, Nitrate, Thiocyanate, and Iodide. Perchlorate, nitrate, thiocyanate, and iodide were analyzed in prepared egg and feed samples by use of ion chromatography−tandem mass spectrometry (IC-MS/MS), as previously described (*27*). A Dionex AS-20 column provided adequate chromatographic separation of perchlorate, nitrate, thiocyanate, and iodide from other matrix components. Two separate MS/MS transitions were monitored to provide qualitative identification for all analyses except iodide. Analytes were quantified on the basis of the ratios of the chromatographic peak areas of analyte and internal standard, as compared with ratios determined by analysis of eight-point calibration curves analyzed before and after each set of samples. Each set of samples was analyzed with characterized quality control material, calibrators, and blanks to ensure overall accuracy and precision of the analysis. All anions were present in feed and water at levels above the method detection limit with the exception of thiocyanate in water, and no contamination problems were observed. Interassay and intra-assay precisions were excellent during the analysis of the study samples, with coefficients of variation of <5% for quality control materials.

Spike Recovery Analysis. Recovery was assessed by adding analytes into six identical 1 g aliquots of lyophilized egg. Three aliquots were suspended separately in 15 mL of filtered water (unspiked), and another three were suspended in 15 mL of multiple-ion spiking solution (containing 5 ng/mL perchlorate, 100 ng/mL iodide, 5000 ng/mL nitrate, and 100 ng/mL thiocyanate). Internal standard solution was added before the sonication to all six aliquots. Another six aliquots were treated similarly, but the internal standard was added after the sonication process. One milliliter of each spiked and unspiked aliquot was assayed for perchlorate, nitrate, thiocyanate, and iodide, respectively. The same spike recovery procedure was used for chicken feed samples. Recoveries ranged from 94 to 104% for eggs and from 93 to 99% for feed.

Egg Consumption and Perchlorate Exposure among U.S. Residents. We further explored the potential significance of eggs as a source of perchlorate exposure by statistical analysis of published data collected from the National Health and Nutrition Examination Survey, 2001−2002 (NHANES) (*28*). NHANES is conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC). This survey is designed to assess the health and nutrition status of the civilian, noninstitutionalized U.S. population. The sampling design for NHANES is based on a complex multistage probability design, which includes selection of primary sampling units (counties) and household segments within the counties, followed by sampling of persons from selected households. Data were collected through a household interview and a standardized physical examination, which was conducted in a mobile examination center. The household interview included a 24 h dietary recall survey, and the physical exam included collection of urine specimens from each participant aged 6 years and older. The 24 h dietary questionnaire included questions about the consumption of eggs and foods containing eggs. NHANES 2001−2002 was conducted in 29 locations throughout the United States, with a random one-third subsample consisting of 2892 NHANES study participants collectively representing the civilian, noninstitutionalized U.S. population, aged 6 years and older. Overall, the survey interview response rate was 83.9% and the exam response rate was 79.6%. Urinary perchlorate, urinary creatinine, and dietary questionnaire data were available for 2820 study participants (*4*). Study participants were categorized on the basis of self-reported egg consumption. To determine the association between egg consumption and urinary perchlorate levels (log transformed) among the 2820 NHANES study participants, we used least-squares means and the p value obtained for the difference of least-squares means (pDiff) generated by the SUDAAN PROC REGRESS model.

Statistical Analysis. For data analysis, we used SAS for Windows (ver. 9.1.3, SAS Institute Inc., Cary, NC) and SUDAAN (ver. 9.0.1, Research Triangle Institute). A nested analysis of variance (PROC GLM) was performed to determine the variability of analytes in eggs between houses and farms. Spearman correlation and regression analysis were performed to determine the relationships between molar amounts of analytes consumed and secreted into eggs by chickens grouped by house. Comparisons of intake and secretion included the following potency factors, based on relative affinities of the anions for human NIS and the assumption of NIS-mediated competitive transport: perchlorate, 1; thiocyanate, 1/15; iodide, 1/30; nitrate, 1/240 (*3*). In humans, thiocyanate potency is enhanced due to a much longer clearance half-life. However, clearance half-lives of these anions are not known in chickens; therefore, we used the unmodified potency factors described by Tonnacchera et al. (*3*). Molar concentrations of

Table 1. Levels of Perchlorate, Nitrate, Thiocyanate, and Iodide Measured in Chicken Water and Feed from Three Different Farms

farm	perchlorate	nitrate	thiocyanate	iodide
		Water (Micrograms per Liter)		
I	0.08 ± 0.16[a]	995 ± 1341	nd[b]	18.18 ± 5.41
II	2.33 ± 0.64	72420 ± 13119	nd	0.16 ± 0.22
III	0.16 ± 0.07	2152 ± 182	nd	0.63 ± 0.19
		Chicken Feed (Micrograms per Kilogram of Dry Weight)		
I	3.72 ± 0.63	4435 ± 1292	467 ± 106	2599 ± 920
II	3.55 ± 0.48	3767 ± 1006	558 ± 109	2921 ± 5889
III	2.93 ± 1.72	6918 ± 2419	337 ± 283	1219 ± 786

[a] Mean ± standard deviation ($n = 5$). [b] Not detected (detection limits were 500, 5.0, 0.10, and 0.025 μg L^{-1} for nitrate, thiocyanate, iodide, and perchlorate, respectively).

each analyte were multiplied by the potency factor and the resulting values for perchlorate, nitrate, and thiocyanate summed as a perchlorate equivalence concentration (PEC). PEC intake was modeled against iodide secretion in eggs to explore a potential competitive transport mechanism for these anions during oogenesis.

RESULTS

We characterized the mean concentration (and standard deviation) of perchlorate and related anions (nitrate, thiocyanate, and iodide) in feed and water at three farms commercially producing eggs in California and Arizona, and we list the data in **Table 1**. Mean perchlorate concentrations ranged from 0.08 to 2.33 μg/L in livestock water samples collected from the three farms. The levels of iodide and nitrate in livestock water varied more than did the levels of perchlorate between the three farms. Thiocyanate was not detected in any water samples.

Table 1 also lists the analyte concentrations found in chicken feed collected at the three farms. The concentrations are expressed as micrograms of perchlorate per kilogram of dry weight. In general, analyte concentrations varied minimally in feed samples collected from the five different chicken houses at each farm. Iodide levels varied significantly within houses on farm II (RSD = 201%), possibly indicating inadequate mixing of feed.

The levels of perchlorate and related anions in water and feed were subsequently used to estimate laying hen intake of these anions, based on average water and feed consumption (*29*). These estimated daily intake data were compared with daily secretion of perchlorate and related anions into eggs. The daily secretion calculations assume that each hen produces one egg each day (*29*). **Table 2** lists the levels of estimated daily intake of perchlorate and related anions by laying hens at each house of each farm. Feed was the primary source of perchlorate, nitrate, thiocyanate, and iodide for these chickens, with water contributing significantly to the intake of perchlorate and nitrate only on farm II.

The mean concentrations (and standard deviations) of perchlorate and related anions found in eggs at the three farms are listed in **Table 3**. The levels of all four analytes were less varied in eggs compared to intake. We also tested seven free-range eggs produced in an area with no known perchlorate contamination in water (feed not analyzed). The mean levels found in the seven free-range eggs were 5.83 (±0.56), 3450 (±520), 156 (±70), and 1900 (±530) μg/kg of dry weight for perchlorate, nitrate, thiocyanate, and iodide, respectively. For all 187 eggs assayed, we found the following levels of analytes (average ± standard deviation): perchlorate, 7.11 ± 1.97 μg/kg of dry weight; nitrate, 2840 ± 2060 μg/kg of dry weight; thiocyanate, 559 ± 432 μg/kg of dry weight; and iodide, 2940 ± 1480 μg/kg of dry weight.

Table 2. Estimated Daily Intake (Micrograms per Hen) of Perchlorate, Nitrate, Thiocyanate, and Iodide by Laying Hens[a]

farm	house	perchlorate	nitrate	thiocyanate	iodide
I	1	0.467	1266	72.2	444
	2	0.446	331	44.2	164
	3	0.527	699	48.8	246
	4	0.411	758	44.7	304
	5	0.291	623	46.8	292
II	6	1.088	20495	50.8	22
	7	1.051	20161	49.0	1504
	8	0.940	19609	63.1	47
	9	0.743	12795	76.2	34
	10	1.065	19560	70.9	25
III	11	0.503	1173	69.5	223
	12	0.453	1117	57.8	67
	13	0.173	1666	4.10	85
	14	0.564	1063	53.6	240
	15	0.144	1497	3.04	64

[a] Estimated daily intake per hen is based on daily feed consumption of 120 g per hen and daily water consumption of 250 mL per hen (*29*).

Table 3. Mean Levels of Perchlorate, Nitrate, Thiocyanate, and Iodide Found in Chicken Eggs (Micrograms per Kilogram of Dry Weight) from Each House

		mean analyte (μg kg^{-1} of dry weight)			
farm	house	perchlorate	nitrate	thiocyanate	iodide
I	1	7.96 ± 1.73[a]	4150 ± 1490	290 ± 76	3000 ± 811
	2	6.93 ± 1.04	2790 ± 1090	294 ± 61	3090 ± 481
	3	6.61 ± 1.23	1250 ± 772	225 ± 46	3030 ± 387
	4	7.08 ± 1.06	1870 ± 730	253 ± 58	3200 ± 577
	5	7.01 ± 2.11	2700 ± 1130	226 ± 62	3290 ± 679
II	6	9.45 ± 1.14	5870 ± 1230	1310 ± 249	2190 ± 513
	7	8.86 ± 1.20	2120 ± 1180	1230 ± 249	3100 ± 842
	8	8.15 ± 1.76	1550 ± 634	1010 ± 352	2740 ± 1086
	9	7.05 ± 1.37	986 ± 687	693 ± 380	1810 ± 428
	10	7.90 ± 1.07	985 ± 800	1020 ± 494	1990 ± 587
III	11	4.68 ± 0.97	553 ± 704	510 ± 77	2740 ± 684
	12	4.19 ± 0.38	3660 ± 528	322 ± 89	1790 ± 247
	13	8.27 ± 1.41	7570 ± 1840	550 ± 113	7340 ± 1850
	14	8.69 ± 1.33	3340 ± 370	571 ± 83	3360 ± 693
	15	4.57 ± 0.68	2860 ± 722	114 ± 103	1980 ± 396

[a] Mean ± standard deviation, $n = 12$ eggs/house.

The perchlorate and thiocyanate data were approximately normally distributed, whereas nitrate and iodide data were skewed to higher values. The thiocyanate levels were bimodal, with the data from farm II (houses 6−10) measurably higher and more variable than those from farms I (houses 1−5) and III (houses 11−15); therefore, two separate analyses were performed: one analysis using farm II data and another analysis using farm I and III data. A log transformation was adequate for achieving approximate normality for iodide but not for nitrate. Using the square root of nitrate values did produce approximately normally distributed results. Nested analysis of variance (ANOVA) models with eggs nested in houses and houses nested in farms indicated that the mean levels in eggs of perchlorate and thiocyanate were significantly different among houses within farms ($p < 0.0001$ for perchlorate; $p < 0.0001$ for thiocyanate in farms I and III; and $p = 0.001$ for thiocyanate in farm II). A similar nested ANOVA of the mean values of the square root of nitrate levels in eggs indicated a significant difference among houses within farms ($p < 0.0001$), and a nested ANOVA of the mean values of the log-transformed iodide levels in eggs indicated a significant difference among

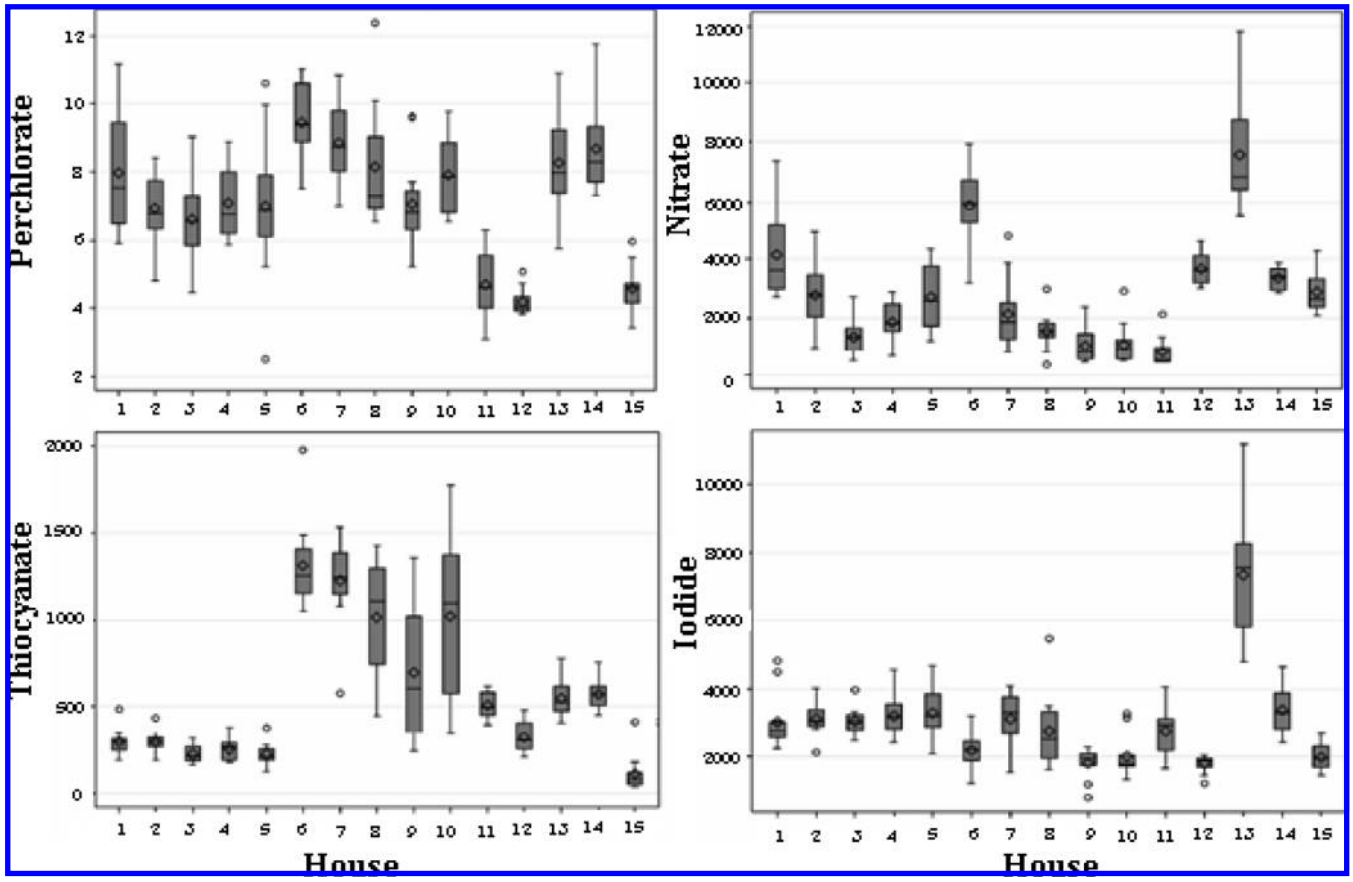

Figure 1. Distribution of perchlorate, nitrate, thiocyanate, and iodide in eggs (μg/kg of dry weight) by house. Box-and-whisker plot indicates the 10th and 90th percentiles (whisker, I), the 25th, 50th, and 75th percentiles (box, —), and (◇) the mean for each analyte. Outliers are plotted as open circles (○). Houses 1−5 are from farm I, houses 6−10 from farm II, and houses 11−15 from farm III.

houses within farms ($p < 0.0001$). Other than the measurably higher and more variable levels of thiocyanate in farm II versus farms I and III as mentioned above and shown in the distribution plots (**Figure 1**), no significant differences were observed for other analytes in eggs among farms ($p = 0.085$ for perchlorate; $p = 0.115$ for thiocyanate farm I versus farm III; $p = 0.525$ for nitrate; and $p = 0.318$ for iodide) for each analyte level by house. The mean analyte levels in eggs between farms were also significantly different ($p < 0.0001$).

For all farms and all houses, the average per egg amounts of perchlorate, nitrate, thiocyanate, and iodide were 0.099, 39.7, 7.83, and 41.1 μg/egg, respectively, based on the edible portion of each egg weighing 55 g (*30*−*32*). By comparing the per egg data with estimated laying hen intake data in **Table 2**, we estimated the mass balance for each analyte. Thus, for chickens producing one egg per day, an average of 23% (SD, ±15%) of perchlorate intake was secreted into the edible portion of egg. Similar mass balance calculations for nitrate, thiocyanate, and iodide indicate average secretion of 3 ± 3.4, 29 ± 45 and 49 ± 45% of intake, respectively.

Table 4 presents the Spearman correlation coefficients found among analytes consumed by chickens and those in eggs. Intake of iodide was positively correlated with egg iodide (0.69, $p = 0.005$). Similarly, intake of perchlorate was positively correlated with egg perchlorate (0.52, $p = 0.046$). Intake and secretion of thiocyanate was also positively correlated, although the correlation was not statistically significant (0.46, $p = 0.081$). There was no association found between intake and average secretion of nitrate by chickens grouped by house. Perchlorate intake was not associated with iodide levels in eggs, whereas intake of thiocyanate was negatively correlated with egg iodide levels

Table 4. Relationship between Intake and Egg Levels of Perchlorate, Iodide, Nitrate, and Thiocyanate by House ($n = 15$)

	analyte in eggs			
analyte intake	perchlorate	nitrate	thiocyanate	iodide
perchlorate	0.52 (0.046)[a]	−0.30 (0.27)	0.81 (0.0002)	−0.35 (0.20)
nitrate	0.51 (0.052)	−0.03 (0.93)	0.77 (0.0008)	−0.43 (0.11)
thiocyanate	0.07 (0.81)	−0.36 (0.19)	0.46 (0.081)	−0.52 (0.048)
iodide	−0.01 (0.97)	0.06 (0.84)	−0.42 (0.12)	0.69 (0.005)

[a] Spearman correlation coefficient (*p* value).

(−0.52, $p = 0.048$). Conversely, both perchlorate intake (0.81, $p = 0.0002$) and nitrate intake (0.77, $p = 0.0008$) were positively correlated with egg thiocyanate levels.

To investigate the potential for competitive transport of analytes into the egg, we modeled analyte intake (molar levels adjusted based on a NIS potency factor) and amount of iodide secreted into eggs. In this observational study we did not control the relative intake levels of nitrate, thiocyanate, iodide, and perchlorate. Bivariate analysis of intake data indicated significant correlation between log10 (nitrate intake) and perchlorate intake ($R^2 = 0.847$). Therefore, we were limited in modeling intake of individual NIS inhibitors versus iodide secretion in eggs and, instead, chose to use PEC intake. Modeling log10 (iodide secretion) versus log10 (PEC intake) yielded a statistically significant ($p < 0.0001$) slope of −0.1257 with 95% CI of (−0.1763, −0.0751). Thus, every 10% increase in PEC intake was associated with a 1.19% (95% CI of 0.71%, 1.67%) decrease in iodide levels in eggs. Because we were limited to observed intake levels, we did not have a uniform representation of PEC levels across the entire range of PEC levels. In fact, the

PEC levels for farm II were about 10 times higher than those in farms I and III. Thus, PEC level and farm were somewhat confounded factors, making it possible that some other factor determined by farm was driving the observed relationship between iodide secretion and PEC level.

On the basis of average perchlorate levels (7.11 μg/kg of dry weight) and average moisture content (74.4%) found in the 187 eggs, we estimated human perchlorate exposure attributable to consumption of these eggs. On a per capita basis, Americans consume an average of 21.8 g of egg per day (*32*). Daily consumption of 21.8 g of the analyzed eggs would lead to daily perchlorate intake of 0.040 μg. By comparison, estimated daily iodide intake from these eggs would be 16.6 μg.

If eggs are a significant source of perchlorate exposure in the U.S. population, then egg consumption would be associated with increased urinary perchlorate levels. We tested for association of egg consumption and urinary perchlorate levels in data published for 2820 NHANES study participants (*28*). The majority of study participants (2510, 89%) reported no egg consumption over the 24 h dietary recall time period, whereas 310 study participants (11%) reported consumption of eggs in the 24 h dietary recall time period. Following categorization by egg consumption, the distribution of urinary perchlorate (μg/L) was compared between these two groups. The least-squares means analysis indicated no association between egg consumption and perchlorate exposure in this population of U.S. residents (pDiff = 0.225).

DISCUSSION

In this paper, we quantified perchlorate and related ion concentrations in eggs and assessed the potential for human (average adult) exposure to perchlorate from egg consumption. We measured perchlorate in all 187 eggs tested, albeit at mean perchlorate levels (0.099 ± 0.0029 μg/egg) much lower than mean iodide levels (41.1 ± 20.7 μg/egg). Consumption of 21.8 g of egg would correspond to 0.040 μg of perchlorate intake per day for an average adult. Average total dietary perchlorate intake is estimated to be 7.2 μg for American adults based on the Total Diet Survey (*11*); thus, perchlorate from eggs is likely to contribute only minimally (0.6%) to total dietary exposure for this population on the basis of these study findings. Similarly, intake of perchlorate from eggs is unlikely to be a significant contributor to overall perchlorate exposure; average perchlorate intake from all sources is estimated to be 10.5 μg/day for American adults based on total urinary excretion of perchlorate (*4*). Consistent with this calculation based on eggs from a localized area, we found no association between egg consumption and perchlorate exposure in 2820 study participants from the NHANES 2001−2002 study (pDiff = 0.225), although only a relatively small percentage of the study participants (11%) reported consuming eggs in the 24 h dietary questionnaire. Additionally, we found average egg iodide levels of (41.1 μg/egg), consistent with previous studies of eggs as a source of iodine (but not perchlorate) intake (*11*). Perchlorate can inhibit iodide transport by competing with iodide at NIS. Because these eggs have 500 times more iodide than perchlorate, the relatively larger amount of iodide could reduce the potential for perchlorate to reduce iodide transport (*3*).

Although our results indicate that eggs are not currently a significant source of perchlorate exposure for the average adult in the United States on the basis of the three farms tested, increased exposure of laying hens to perchlorate through contaminated feed or water may increase perchlorate levels in eggs. Pena et al. (*24*) found significant perchlorate accumulation in developing and fully formed eggs produced by chickens following an intramuscular injection of radioactive perchlorate. In the present study, perchlorate levels in eggs were positively correlated with total perchlorate intake from water and feed ($p < 0.001$), underscoring the importance of both water and feed as potential exposure sources. In fact, perchlorate levels in water were not correlated with perchlorate levels in eggs, likely due to the varied contribution of perchlorate from feed.

On average, the laying hens were estimated to secrete 23% of total perchlorate intake into eggs, assuming that the hens laid one egg per day. Iodide and thiocyanate also appear to be concentrated in eggs, with iodide transported most efficiently (49%) into eggs. The mechanism of perchlorate, thiocyanate, and iodide movement from intake to egg is not characterized, although the efficiency of anion transport into eggs is consistent with an active transport mechanism. These data are not necessarily consistent with transport by NIS. On the basis of human data for the relative affinities of these anions for NIS (*3*) and the relatively long physiological half-life of thiocyanate, we would expect thiocyanate and perchlorate to be transported more efficiently than iodide. From our regression analysis we found that PEC intake was associated with decreased iodide levels in eggs. These data are consistent with a transmembrane protein actively transporting iodide (and also perchlorate, thiocyanate, and nitrate) during egg formation. This competitive transport mechanism would also explain why the percent secretion for iodide, perchlorate, and nitrate is lowest for eggs from hens consuming the highest amounts of these three potentially related analytes. Our data indicate that laying hens can concentrate perchlorate and related anions from feed or water into eggs, although the mechanism of this action is unknown. A subsequent controlled-exposure study would be required to delineate the relative potential impact of perchlorate, nitrate, and thiocyanate on iodide levels in eggs.

Nitrate levels in water from farm II were relatively high: 63% above the human drinking water maximum contaminant level of 44,300 μg/L and 34−73 times higher than water nitrate levels at farms III and I, respectively. Despite the elevated water nitrate levels at farm II, eggs produced there contained nitrate levels similar to eggs produced at the other two farms. Therefore, nitrate may not accumulate in eggs in response to increased nitrate intake. This lack of correlation between nitrate intake and excretion/secretion may be due to endogenous formation and/or metabolism of nitrate.

Thiocyanate levels in eggs differed between farms (**Figure 1**), even though the levels in feed and water were not different between farms. One explanation for this finding is that the farms used different feeds containing different thiocyanate precursors. Many foods such as almonds, cassava, and cruciferous vegetables contain chemicals (e.g., cyanide, glucosinolates, isothiocyanates) that are metabolized to form thiocyanate in vivo. We did not assay the feed and water for these thiocyanate precursors that could have led to higher egg thiocyanate levels after consumption and metabolism.

Trace levels of iodide were found in all farm water samples tested. The range of iodide in farm water in our study (ND−24.6 μg/L) is consistent with previously published iodine levels in the western United States (0.5−66 μg/L) (*33*). Potential sources of iodine in rivers include oceanic cyclic iodine that is delivered to river watersheds atmospherically and iodine weathered from soils and rocks (*33*). Notably, chickens consuming the water containing the highest mean iodide level (18.18 μg/L) received only ∼1.5% of their total iodine intake from the water, with much higher amounts coming from the iodine-fortified feed.

Feed was the primary source of perchlorate, nitrate, thiocyanate, and iodide intake for these chickens, with water contributing >50% of the total intake of perchlorate and nitrate only on farm II. Perchlorate in water from farm II (2.33 μg/L) was expected because this farm used water from the lower Colorado River; however, the relative importance of feed as a source of perchlorate intake was higher than expected. This background of perchlorate intake by livestock through feed is consistent with analysis of dairy cattle perchlorate intake (*18, 23*). Livestock intake of environmental toxicants can lead to human exposure, especially if the environmental toxicant is concentrated into food products. Our finding of possible transport of perchlorate, thiocyanate, and iodide into eggs merits further investigation.

In conclusion, although perchlorate levels in chicken eggs are positively correlated with perchlorate intake by chickens, we find no evidence based on the 15 chicken houses and 3 farms tested that eggs are a significant dietary source of perchlorate exposure to average American adults. Additionally, eggs are a potential source of iodine, which can modulate perchlorate action at NIS. The association of PEC intake with decreased iodide levels in eggs is consistent with a transmembrane protein competitively transporting iodide (and also perchlorate, thiocyanate, and nitrate) during egg formation.

ABBREVIATIONS USED

ClO_4, perchlorate; I, iodide; IC-MS/MS, ion chromatography−tandem mass spectrometry; NHANES, National Health and Nutrition Examination Survey; NIS, sodium−iodide symporter; NO_3, nitrate; PEC, perchlorate equivalence concentration; SCN, thiocyanate.

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