J O U R N A L O F
AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008**, *56*, 5443–5450 **5443**

# Perchlorate in the Feed–Dairy Continuum of the Southwestern United States

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Perchlorate has the potential to cause thyroid dysfunction by inhibiting iodide uptake by the sodium iodide symporter. Perchlorate-contaminated waters may lead to human exposure through drinking water and food chain transfer in crops by way of irrigation water. Perchlorate has been found in dairy milk collected nationally and internationally. This study was conducted to evaluate perchlorate in the feed-dairy continuum in the southwestern United States. All feed products collected at dairies in this study had detectable levels of perchlorate as analyzed by ion chromatography–tandem mass spectrometry. The calculated total perchlorate intake across dairies ranged from 1.9 to 12.7 mg/cow per day. The variation in total perchlorate intake across dairies was largely associated with variation in forage and silage products. Alfalfa products were the single most important source of perchlorate intake variability among dairies. The estimated perchlorate intake from drinking water ranged from 0.01 mg per cow per day and was generally less than 2% of the total perchlorate intake. The perchlorate content of milk ranged from 0.9 to 10.3 μg/L and was similar to levels reported by the Food and Drug Administration's Total Diet Study. The perchlorate content of milk was significantly related to the presence of perchlorate in feed but the variation of perchlorate in milk could not be explained by feed intake alone.

**KEYWORDS: Perchlorate; milk; dairy feed**

## INTRODUCTION

Perchlorate has been discovered in surface and groundwater supplies throughout the United States (U.S.). There is concern that perchlorate contamination of waters can lead to human exposure to perchlorate through drinking water and food chain transfer in feed and food crops by way of irrigation water. Indeed, a recent study of the U.S. population found perchlorate exposure in all 2820 people tested (*1*). Perchlorate has the potential to cause thyroid dysfunction by inhibiting iodide uptake by the sodium iodide symporter (NIS) (*2*). A reference dose (RfD) of 0.7 μg/kg per day has been established by the U.S. Environmental Protection Agency (*3*). This action followed review of a human dose–response study (*4*) and a recommendation by the National Academy of Science (*5*) that inhibition of iodine uptake by the thyroid in humans was a key biochemical event that preceded any health effects caused by perchlorate. Thus the RfD is based upon a nonadverse effect rather than an "adverse effect as the point of departure for the perchlorate risk assessment (*5*)". One epidemiological study examined perchlorate exposure in iodine replete Chilean women and found no changes in thyroid hormone levels despite exposure doses estimated to be higher than the RfD (*6*). However, another recent study found that estimated perchlorate doses below the reference dose were associated with altered thyroid hormone levels in women with low iodine intake (*7*). One explanation of these different findings is that Tellez et al. (*6*) examined only three women with average urinary iodine <100 μg/L, while Blount et al. (*7*) examined 348 women with urinary iodine <100 μg/L. These studies underscore the importance of understanding sources of iodine intake and perchlorate exposure. Increased iodine intake could decrease the ability of a given dose of perchlorate to inhibit iodide transport.

Several plant species have been shown to absorb perchlorate from soil and irrigation water (*8*) and there is evidence that perchlorate accumulates in forage, grain, edible fruit, and vegetables, and can be found in dietary supplements (*9–13*). Studies have found perchlorate in women's breast milk (*14, 15*) and dairy milk collected nationally (*14, 16, 17*) and internation-

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10.1021/jf0733923 CCC: $40.75 
Published on Web 06/13/2008

ally (*18, 19*). Further, dairy products have been associated with human exposure through biomonitoring (*20*), and infants, in particular, may be exposed to perchlorate through milk (*21*). However, milk is also a significant source of iodine intake in the U.S. population (*22*). The mechanism of concentration of iodine by the mammary gland is similar to that of the thyroid (*23*).

One experimental evaluation, which introduced perchlorate into dairy cows through feed, water, and infusion, found that the perchlorate content in milk was significantly correlated with perchlorate intake by dairy cows (*24*). However, up to 80% of the dietary perchlorate was metabolized by the cow, presumably in the rumen, reducing the transfer of perchlorate to milk. It has been demonstrated that active transport processes are involved in the transfer of perchlorate across the blood−mammary gland interface (*25*).

Perchlorate contamination in the Colorado River is well documented and is introduced into Lake Mead through the Las Vegas Wash as a result of contamination from former perchlorate manufacturing activities. It has been reported that the Colorado River below Lake Mead has had perchlorate concentrations ranging from 2 to 9 $\mu$g/L (*10, 26*) and perchlorate has been consistently found in food crops produced in the lower Colorado River region (LCRR) (*10–12*). A substantial number of feed and forage crops utilized in western animal husbandry industries are produced in the LCRR. For example, over 85 000 ha of alfalfa produced in this region is shipped throughout the western United States (*27, 28*). The objective of this study is to evaluate perchlorate in the feed−dairy continuum of the southwestern United States.

## MATERIALS AND METHODS

**Sample Collection.** Field samples were collected in the LCRR to evaluate the potential for perchlorate accumulation in forage crops used by animal husbandry industries. These samples were collected from 2005 through 2006. All fields selected for sampling were irrigated with water from the Colorado River and were collected from the Imperial Valley of California and the lower Colorado River Valley of California and Arizona. For comparative purposes we also collected alfalfa samples in the Rio Grande Valley from Albuquerque, New Mexico to El Paso, Texas in the summer of 2006. In these areas we also collected accompanying irrigation water samples in a nearby canal or in the main channel of the Rio Grande River.

Although very few dairies are physically located in the LCRR, many dairies in the western U.S. utilize feed and forage produced in the lower Colorado River region. In addition, many dairies in the southwestern U.S. are more directly impacted by Colorado River water transported outside the Colorado River region in the Central Arizona Project (CAP) and California Aqueduct systems. During the summer of 2006 we sampled 33 distinct dairy farm operations in Arizona and southern California (**Table 1** and **Figure 1**). The sampling was restricted to the summer months to minimize variability that might be associated with seasonal variation in milk production.

Our first step in sampling each dairy operation was visiting the headquarters and obtaining a description of the feed ration for the high milk producing cows. Our assumption was that most milk in the tanks would be from the high milk producers. In most cases the ration sheets were provided to us as a computer print out often formulated by a nutritional consultant. These are typically composed considering the animal's nutritional requirement as influenced by milk production and environmental stress and commodity costs and availability. The next step was visiting the commodity barn and collecting a random sample of each component of the feed ration. For all dairy operations, we sampled forage and silage feed components separately. Many dairies prepare a premix consisting of all, or a subset of, grain products, protein, fat, and mineral supplements to reduce the potential for costly errors in preparing the final feed blend. Where dairies prepared a premix, only this mixture was sampled, instead of the individual components,

**Table 1.** The Dairy Breed, Location, and Date of Sampling for Feed, Water, and Milk Samples

| dairy | breed | area (county/state) | date sampled |
|---|---|---|---|
| 1 | Holstein | Pinal, AZ | June 14, 2006 |
| 2 | Holstein | Pinal, AZ | June 14, 2006 |
| 3 | Holstein | Pinal, AZ | June 14, 2006 |
| 4 | Holstein | Pinal, AZ | June 14, 2006 |
| 5 | Holstein | Pinal, AZ | June 14,2006 |
| 6 | Holstein | Pinal, AZ | June 14,2006 |
| 7 | Holstein | Pinal, AZ | June 14, 2006 |
| 8 | Holstein | Pinal, AZ | June 14, 2006 |
| 9 | Holstein | Maricopa, AZ | June 21, 2006 |
| 10 | Holstein | Maricopa, AZ | June 21, 2006 |
| 11 | Jersey | Maricopa, AZ | June 21, 2006 |
| 12 | Holstein | Maricopa, AZ | June 21, 2006 |
| 13 | Holstein | Maricopa, AZ | June 21, 2006 |
| 14 | Holstein | Maricopa, AZ | June 21, 2006 |
| 15 | Holstein | Maricopa, AZ | June 21, 2006 |
| 16 | Jersey | Maricopa, AZ | June 21, 2006 |
| 17 | Holstein | Maricopa, AZ | June 21, 2006 |
| 18 | Holstein | Maricopa, AZ | June 21, 2006 |
| 19 | Holstein | Pima, AZ | July 15, 2006 |
| 20 | Holstein | Pima, AZ | Aug 8, 2006 |
| 21 | Holstein | Maricopa, AZ | Aug 31, 2006 |
| 22 | Holstein | Maricopa, AZ | Aug 31, 2006 |
| 23 | Holstein | Maricopa, AZ | Aug 31, 2006 |
| 24 | Holstein | Maricopa, AZ | Aug 31, 2006 |
| 25 | Holstein | Maricopa, AZ | Aug 31, 2006 |
| 26 | Holstein | Pinal, AZ | Sept 20, 2006 |
| 27 | Holstein | Riverside, CA | Sept 27, 2006 |
| 28 | Holstein | Riverside, CA | Sept 27, 2006 |
| 29 | Holstein | Riverside, CA | Sept 27, 2006 |
| 30 | Holstein | San Bernardino, CA | Sept 27, 2006 |
| 31 | Holstein | San Bernardino, CA | Sept 27, 2006 |
| 32 | Holstein | Riverside, CA | Sept 27, 2006 |
| 33 | Holstein | Imperial, CA | Sept 27, 2006 |

due to our budget limitations. This decision was also rationalized due to previous observations that perchlorate accumulated to much lower levels in grain and seed crops as compared to leafy crops (*9, 12, 17, 29*). For the cases in which dairies did not prepare a premix, we sampled all components of the feed ration individually.

For the next step, we proceeded to the milking parlor where we obtained a milk sample from the main tank by either using a sampling ladle or faucet/septum sampling port located on the tank. It should be noted that all feed, water, and milk samples were collected personally by the senior author with three exceptions. For three dairies (dairies 1, 16, and 24), the milk tank had recently been drained by a transport truck operated by the contracted milk processing facility. For these three dairies we asked local volunteers to collect the milk samples and ship them to our laboratory. Followup inquiry was required before we received these samples and two (dairies 1 and 24) yielded suspect results. As explained later, milk from these two dairies was eventually excluded from the final evaluation. The cattle drinking water was also sampled. In most cases the drinking water used by the herd could be sampled from a washing sink in the milking parlor.

**Sample Processing.** All of the samples we collected were transported to our laboratory within 24 h in ice chests. The milk samples, water samples, and liquid feed components (molasses and whey) were frozen until analysis. The solid feed samples were weighed as fed and dried in an oven at 65 °C for 48 h before dry weights were determined. These data were used to calculate perchlorate concentrations at moisture contents as fed and on a dry weight basis. All solid feed samples were ground for analysis and stored in vials at room temperature away from direct light.

For feed products, we used an extraction procedure where 600 mg of oven-dried product was weighed into centrifuge tubes, 15 mL of deionized (DI) water was added, the tubes were boiled for 30 min, and the contents were placed in a refrigerator overnight with occasional gentle shaking (*30*). The tubes were then centrifuged for 30 min and the supernatants filtered through 0.2-$\mu$m Gel-man ion membrane syringe filters. Two milliliters of the above extract (extract one) was reacted

**Table 2.** Perchlorate content of selected forage crops in the lower Colorado River region (LCRR) and alfalfa in the Rio Grande Valley (RGRV)

| | | | perchlorate | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | dry weight (μg/kg) | | | as hay fed (μg/kg) | | |
| crop | area | n[a] | min[b] | max[c] | mean | min[b] | max[c] | mean |
| Alfalfa hay (*Medicago sativa*) | LCRR | 28 | 221 | 1670 | 844 | 188 | 1420 | 709 |
| Alfalfa hay | RGRV | 15 | 65 | 774 | 280 | 55 | 658 | 228 |
| Bermuda grass (*Cynodon dactylon*) | LCRR | 22 | 43 | 2196 | 261 | 36 | 1844 | 219 |
| Klein grass (*Panicum coloratum*) | LCRR | 7 | 229 | 723 | 426 | 195 | 614 | 362 |
| Sudan grass (*Sorghum vulgare var. sudanense*) | LCRR | 17 | 130 | 1032 | 491 | 117 | 928 | 441 |

[a] Number of samples. [b] Minimum. [c] Maximum.

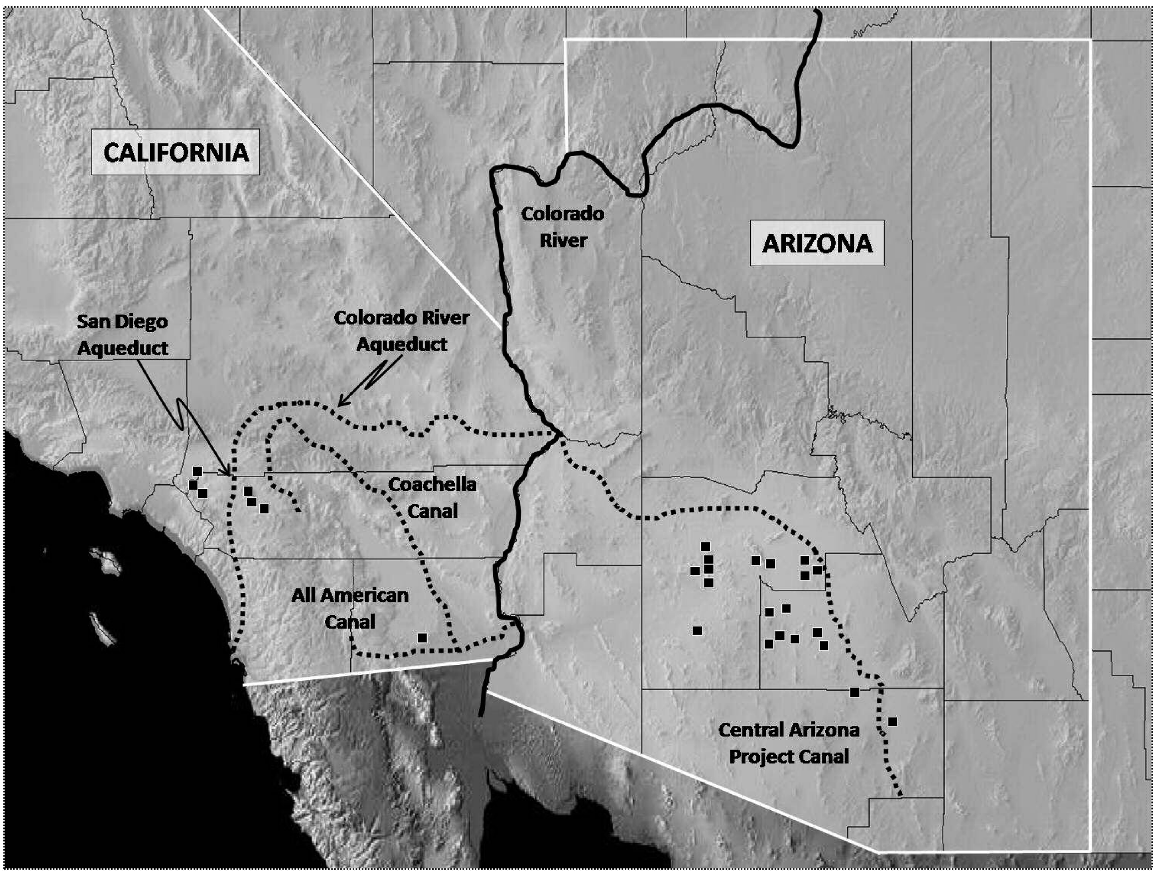


**Figure 1.** Locations of dairies sampled in southwestern United States. The symbols may represent one or more (if in close proximity) dairies.

with 1000 mg DD-alumina. Vials were gently agitated two or three times over a 24-h period. Eighteen milliliters of DI water was then added to this mixture. After stirring and settling, this solution was filtered through another 0.2-μm Gel-man ion membrane syringe filter and the resulting solution was labeled "extract 2". This sample was stored in the freezer until analysis.

**Perchlorate Analysis.** Perchlorate contents in drinking water, feed extracts, and milk extracts were determined by ion chromatography/tandem mass spectroscopy (IC-MS/MS), using the stable isotope-labeled internal standard methodology reported previously (*31*). Water samples and feed extracts (0.5 mL) were spiked with isotopically labeled internal standards ($Cl^{18}O_4^-$, $SC^{15}N^-$) and diluted 1:1 with DI water. Milk samples (0.5 mL) were spiked with stable isotope labeled perchlorate ($Cl^{18}O_4^-$) and thiocyanate ($SC^{15}N^-$). Milk proteins were subsequently precipitated by addition of 3 mL of cold ethanol (−20 °C). Samples were centrifuged (3016 g, −5 °C) for 35 min. Supernatant was transferred to a clean centrifuge tube and evaporated to dryness under a stream of nitrogen at 60 °C. The sample was resuspended in 1.0 mL of DI water and added to a preconditioned C18 solid-phase extraction cartridge. The breakthrough fraction and a subsequent 1 mL wash of DI water were pooled and spiked with 3-chlorobenzenesulfonate for use as an internal standard for iodide. Subsequently, 1 mL of this solution was transferred to an autosampler vial for IC-MS/MS analysis.

Perchlorate and thiocyanate were quantified based on the peak area ratio of analyte to stable isotope-labeled internal standard, while iodide was quantified based on the peak area ratio of iodide to 3-chlorobenzenesulfonate. A subset of samples (10%) were analyzed further, using standard addition, and produced acceptable percent differences of <10%. Absolute assay accuracy was verified by the blind analysis of four different reference solutions containing perchlorate (AccuStandard, New Haven, CT); analysis of these proficiency testing solutions across the study time period yielded an average percent difference of −5.2%. The perchlorate method detection limit was estimated to be 0.02 μg/L and the minimum reporting limit (MRL) was 0.1 μg/L. All feed products and milk had perchlorate levels above the MRL by IC-MS/MS. Previous work has shown iodine in milk is largely in the iodide form (*32*).

For the determination of nitrate in milk we used an extraction procedure developed at the U.S. Environmental Protection Agency laboratory in Athens, GA (personal communication). Fifteen millilieters of milk was added to centrifuge tubes and boiled for 10 min. After boiling, 5 drops of acetic acid was added to the mixture, the tubes were then centrifuged for 30 min, and the supernatants were filtered. The nitrate content of this milk extract solution was determined by using Griess-Ilosovay method after reduction with copperized cadmium (*33*), using an ALPKEM RFA2 automated colorimeter (OI Analytical, College Station, TX).

There were four samples of drinking water that had perchlorate levels below the MRL and for these we assigned values of 0.05 μg/L perchlorate for intake estimates. One dairy in our survey was located in a highly regulated water management area and was required to compile quantitative estimates of water utilized. We used water consumption values of 170 L per cow per day, as measured at this dairy, to estimate perchlorate intake from drinking water.

Estimates of perchlorate intake in each component of the feed ration were calculated from measured perchlorate concentrations in each component of the ration and the quantity fed per cow for the high milk producers as reported in the ration sheets obtained from each dairy. Intake values were calculated on a dry weight basis. Perchlorate intake by water was estimated from measured concentrations and an estimated consumption of 170 L day. All statistical analysis was performed with the SAS/STAT software package, version 8 (*34*).

## RESULTS AND DISCUSSION

**Perchlorate Content in Feed.** The perchlorate content of selected forage crops collected in fields irrigated with lower Colorado River water ranged from 43 to 2196 μg/kg on a dry weight (dw) basis (**Table 2**). The concentration of perchlorate in alfalfa (*Medicago sativa*) hay ranged from 221 to 1670 μg/kg dw and was generally higher than other forage products produced in the lower Colorado River region. The perchlorate concentration of alfalfa produced in the Rio Grande Valley ranged from 65 to 774 μg/kg dw. The perchlorate content of irrigation water in the LCRR ranged from 2 to 5 μg/L during the sampling period. These perchlorate levels are lower than previously reported studies, most likely due to effective remediation of perchlorate-contaminated groundwater near the Las Vegas Wash (*35*). The perchlorate content of irrigation water in the Rio Grande Valley was consistently below detection by IC-MS/MS (<0.05 μg/L). Previous work (*9*) reported a perchlorate bioconcentration factor from irrigation water to alfalfa of 380 (μg/kg fw/μg/L). Fresh alfalfa is approximately 25% dry matter, which would convert the bioconcentration ratio to 1500 (μg/kg dw/μg/L) on a dry weight basis. Thus, alfalfa could potentially accumulate perchlorate in the range observed in the Rio Grande Valley from irrigation water with 0.05 μg/L perchlorate. Alternatively, perchlorate in alfalfa could also originate from other sources such as soil and fertilizer. Perchlorate forms naturally in the atmosphere and is deposited in trace levels onto soil (*36*). Perchlorate can accumulate in arid regions and then be mobilized by irrigation (*37*).

The amounts of various feed products fed varied by dairy (**Table 3**). The amount of total feed used ranged from 14 to 28 kg dw/cow but more often was in the 20 to 25 kg dw/cow range. With the exception of vegetable/fruit waste, whey, and molasses, all nonforage and silage products may or may not have been included in a premix. For example, in some instances cotton seed (*Gossypium* sp) or corn (*Zea mays*) was included in the premix and, in other instances, it was not. Fruit and vegetable waste included carrot culls (*Daucus carota sativus*), potato culls (*Solanum tuberosum*), melon waste (*Cucumis melo* and *Citrullus lanatus*), and citrus pulp (*Citrus* sp.). All feed products collected at dairies in this survey had detectable levels of perchlorate by IC-MS/MS (**Table 4**). Perchlorate content was generally higher in forage and silage products. One noteworthy finding was the consistency of detectable levels observed in grain products. Previous work had shown generally lower perchlorate accumulation in grain products, often below detection by IC-MS/MS (*12, 17*).

All dairies sampled used alfalfa hay, and the mean perchlorate content was 383 μg/kg dw. One dairyman volunteered that his concern about perchlorate prompted him to have his alfalfa hay shipped in from Utah. However, the alfalfa hay at this dairy contained perchlorate at a concentration of 693 μg/kg dw. These data collectively show the propensity of alfalfa to bioconcentrate perchlorate whether grown within or outside the Colorado River region. Perchlorate accumulation is related to crop transpiration (*38*) and leafy crops such as alfalfa are known to have high transpiration rates (*39*). Previous work has shown that leafy vegetables, such as lettuce (*Lactuca sativa*), also accumulate perchlorate whether grown within or outside the lower Colorado River region (*10, 29*). Alfalfa production systems also rely almost exclusively on symbiotic nitrogen fixation for the plant's nitrogen nutritional requirement. Previous work has suggested that nitrate may compete with perchlorate for plant uptake (*40–42*) and lower fertilizer-derived nitrate in the crop rooting zone may partially account for the generally higher perchlorate concentrations observed in alfalfa.

**Table 3.** Feed Intake by Sources Across Dairies Surveyed

| | | dry weight feed (kg/cow) | | | |
|---|---|---|---|---|---|
| crop | no. | min[c] | max[d] | mean | median |
| alfalfa hay | 33 | 1.28 | 13.09 | 4.90 | 3.53 |
| Bermuda hay | 3 | 0.34 | 1.01 | 0.57 | |
| oat hay (*Avena sativa*) | 1 | | | 0.25 | |
| alfalfa green chop | 14 | 0.53 | 8.36 | 4.06 | 3.34 |
| alfalfa silage | 15 | 0.30 | 6.94 | 2.65 | 2.06 |
| corn silage (*Zea mays*) | 27 | 0.48 | 10.30 | 3.94 | 3.52 |
| oat silage | 1 | | | 2.94 | |
| sorghum silage (*Sorghum bicolor*) | 2 | 2.13 | 3.18 | 2.66 | |
| wheat silage (*Triticum aestivum*) | 3 | 0.06 | 2.62 | 1.25 | |
| vegetable/fruit waste | 5 | 0.38 | 1.53 | 0.84 | 0.81 |
| dried beet pulp (*Beta vulgaris altissima*) | 2 | 0.89 | 1.49 | 1.19 | |
| premix[a] | 25 | 2.43 | 14.55 | 9.40 | 10.54 |
| corn grain products | 19 | 1.12 | 6.28 | 3.33 | 2.85 |
| barley products (*Hordeum vulgare*) | 2 | 2.81 | 3.07 | 2.94 | |
| distillers grain | 5 | 0.12 | 2.13 | 0.91 | 0.82 |
| malt | 1 | | | 0.56 | |
| bakery waste | 2 | 0.71 | 1.15 | 0.93 | |
| mill run | 1 | | | 2.02 | |
| cotton seed (*Gossypium sp.*) | 12 | 0.73 | 3.45 | 2.20 | 2.30 |
| canola product (*Brassica napus*) | 4 | 0.24 | 4.88 | 1.80 | 1.04 |
| soy product (*Glysine max*) | 2 | 0.42 | 1.68 | 1.05 | |
| almond hulls (*Prunus domestica*) | 2 | 0.77 | 1.99 | 1.31 | |
| bypass fat | 6 | 0.15 | 0.48 | 0.27 | 0.25 |
| mineral supplements | 7 | 0.15 | 1.51 | 0.59 | 0.51 |
| molasses | 7 | 0.34 | 1.13 | 0.64 | 0.45 |
| whey | 5 | 0.76 | 4.54 | 2.87 | 2.72 |
| **total[b]** | **33** | **14.0** | **27.66** | **22.38** | **23.05** |

[a] Many dairies prepare a premix, which often includes all or a subset of grain, protein supplements, fat, and mineral supplements. For dairies that prepared a premix, the mixture instead of individual components was sampled. For dairies that did not prepare a premix, all components of the feed ration were sampled individually. [b] These mean and range values were obtained after totaling feed intake at each dairy. These values cannot be calculated from the means in the rows above because with the exception of alfalfa hay only subsets of the products listed were fed at any one dairy. [c] Minimum. [d] Maximum.

**Estimated Perchlorate Intake As Feed and Water.** The calculated total perchlorate intake across dairies ranged from 1.9 to 12.7 mg/cow per day (**Table 5**). The variation in total perchlorate intake across dairies was largely associated with variation in forage and silage products. The perchlorate intake as alfalfa product (hay, green chop, and silage) ranged from 0.7 to 9.0 mg/cow per day and regression analysis showed alfalfa accounted for 94% of the variation in total perchlorate intake. The perchlorate intake from all forage and silage products combined ranged from 0.9 to 11.8 mg/cow per day and explained 97% of the variation in total perchlorate intake by simple regression analysis. The contribution of feed products other than forages and silage ranged from 0.5 to 2.2 mg/cow

**Table 4.** Perchlorate Content of Feed Sources Collected from Dairies Surveyed

| | | perchlorate concn by source | | | | | |
|---|---|---|---|---|---|---|---|
| | | dry weight (μg/kg) | | | as fed (μg/kg or μg/L) | | |
| source | n[b] | min[c] | max[d] | mean | min[c] | max[d] | mean |
| alfalfa hay (μg/kg) | 33 | 106 | 897 | 383 | 83 | 815 | 322 |
| Bermuda hay (μg/kg) | 3 | 126 | 407 | 227 | 112 | 372 | 204 |
| oat hay (μg/kg) | 1 | | | 113 | | | 63 |
| alfalfa green chop (μg/kg) | 14 | 68 | 1778 | 764 | 13 | 633 | 218 |
| alfalfa silage (μg/kg) | 15 | 42 | 782 | 297 | 12 | 368 | 144 |
| corn silage (μg/kg) | 27 | 66 | 675 | 204 | 19 | 229 | 72 |
| oat silage (μg/kg) | 1 | | | 317 | | | 128 |
| sorghum silage (μg/kg) | 2 | 98 | 787 | 442 | 26 | 284 | 155 |
| wheat silage (μg/kg) | 3 | 97 | 245 | 161 | 44 | 118 | 77 |
| vegetable/fruit waste (μg/kg) | 5 | 98 | 270 | 161 | 11 | 34 | 22 |
| dried beet pulp (μg/kg) | 2 | 105 | 111 | 108 | 86 | 96 | 91 |
| premix[a] (μg/kg) | 25 | 40 | 142 | 85 | 37 | 125 | 75 |
| corn grain products (μg/kg) | 19 | 13 | 297 | 87 | 11 | 248 | 76 |
| barley products (μg/kg) | 2 | 98 | 107 | 102 | 80 | 88 | 84 |
| distillers grain (μg/kg) | 5 | 64 | 177 | 104 | 22 | 161 | 85 |
| malt (μg/kg) | 1 | | | 93 | | | 30 |
| bakery waste (μg/kg) | 2 | 55 | 61 | 58 | 49 | 52 | 50 |
| mill run (μg/kg) | 1 | | | 83 | | | 77 |
| cotton seed (μg/kg) | 12 | 42 | 118 | 80 | 39 | 106 | 73 |
| canola product (μg/kg) | 4 | 72 | 102 | 87 | 65 | 99 | 80 |
| soy product (μg/kg) | 2 | 85 | 96 | 91 | 79 | 90 | 85 |
| almond hulls (μg/kg) | 2 | 75 | 517 | 244 | 66 | 409 | 198 |
| bypass fat (μg/kg) | 6 | 41 | 216 | 90 | 39 | 198 | 85 |
| mineral supplements (μg/kg) | 7 | 13 | 110 | 75 | 11 | 105 | 68 |
| molasses (μg/kg) | 7 | | | | 38 | 88 | 61 |
| whey (μg/L) | 5 | | | | 20 | 56 | 42 |
| water (μg/L) | 33 | | | | <MRL | 4.1 | 0.6 |

[a] Many dairies prepare a premix, which often includes all or a subset of grain, protein supplements, fat, and mineral supplements. For dairies that prepared a premix, the mixture instead of individual components was sampled. For dairies that did not prepare a premix, all components of the feed ration were sampled individually. [b] Number of samples. [c] Minimum. [d] Maximum.

per day and did not significantly ($P > 0.20$) affect variation in total perchlorate intake. For the two dairies where the total daily intake exceeded 10 mg/cow per day, the variation was largely associated with the perchlorate content of the alfalfa green chop. The intakes of perchlorate in alfalfa green chop in these two dairies were 5.6 and 8.6 mg/cow per day. Many dairy feed products, including alfalfa hay, are often transported from more than 100-mile distances. However, products such as green chop and silage, which have a high moisture content, are generally produced locally. The two dairies with the high green chop perchlorate values were located in a region of central Arizona where irrigated lands are known to use Colorado River water delivered via the Central Arizona Project Aqueduct system. Previous work with lettuce (*10*) has shown that perchlorate concentrations in plants decline with age. Alfalfa for green chop is harvested almost daily as needed, regardless of maturity, and it is possible that immature alfalfa used as green chop would have elevated levels of perchlorate.

The perchlorate intakes from feed observed in a previous study conducted at Beltsville, MD (*24, 43*) ranged from 0.43 to 0.46 mg/cow per day. Although the perchlorate concentration of alfalfa in this previous study (*43*) was 228 μg/kg dw, and within the range of those we observed in our survey, alfalfa was only 3% of the total feed ration on a dry weight basis. In our survey, the percentage alfalfa in the feed ration, on a dry weight basis, ranged from 29 to 86% across the dairies we surveyed, accounting for the larger impact of alfalfa.

The perchlorate content of cattle drinking water ranged from below the MRL (<0.05 μg/L) to 4.1 μg/L. Most of the drinking water used at these dairies was groundwater and it is difficult

**Table 5.** Perchlorate Intake by Feed Sources Across Dairies Surveyed

| | | perchlorate intake (mg/cow)[b] | | | |
|---|---|---|---|---|---|
| | no. | min[d] | max[e] | mean | median |
| alfalfa hay | 33 | 0.27 | 6.98 | 1.70 | 1.15 |
| Bermuda hay | 3 | 0.05 | 0.15 | 0.11 | |
| oat hay | 1 | | | 0.03 | |
| alfalfa green chop | 14 | 0.19 | 8.62 | 2.70 | 1.93 |
| alfalfa silage | 15 | 0.02 | 3.18 | 0.76 | 0.60 |
| corn silage | 27 | 0.05 | 2.80 | 0.60 | 0.42 |
| oat silage | 1 | | | 0.93 | |
| sorghum silage | 2 | 0.31 | 1.68 | 0.99 | |
| wheat silage | 3 | 0.01 | 0.37 | 0.21 | |
| vegetable/fruit waste | 5 | 0.05 | 0.27 | 0.13 | 0.13 |
| dried beet pulp | 2 | 0.10 | 0.16 | 0.13 | |
| premix[a] | 25 | 0.23 | 1.92 | 0.81 | 0.78 |
| corn grain products | 19 | 0.06 | 0.79 | 0.28 | 0.22 |
| barley products | 2 | 0.30 | 0.30 | 0.30 | |
| distillers grain | 5 | 0.01 | 0.15 | 0.09 | 0.07 |
| malt | 1 | | | 0.05 | |
| bakery waste | 2 | 0.04 | 0.07 | 0.05 | |
| mill run | 1 | | | 0.09 | |
| cotton seed | 12 | 0.05 | 0.29 | 0.18 | 0.18 |
| canola product | 4 | 0.02 | 0.41 | 0.15 | 0.09 |
| soy product | 2 | 0.04 | 0.16 | 0.10 | |
| almond hulls | 2 | 0.11 | 0.60 | 0.29 | |
| bypass fat | 6 | 0.01 | 0.10 | 0.03 | 0.02 |
| mineral supplements | 7 | 0.01 | 0.17 | 0.05 | 0.04 |
| molasses | 7 | 0.01 | 0.09 | 0.04 | 0.03 |
| whey | 5 | 0.04 | 0.14 | 0.09 | 0.08 |
| water | 33 | 0.01 | 0.70 | 0.10 | 0.06 |
| **total[c]** | **33** | **1.85** | **12.66** | **4.88** | **4.00** |

[a] Many dairies prepare a premix, which often includes all or a subset of grain, protein supplements, fat, and mineral supplements. For dairies that prepared a premix, the mixture instead of individual components, was sampled. For dairies that did not prepare a premix, all components of the feed ration were sampled individually. [b] These values were calculated as the mean of the multiples from all data and are often slightly different than the multiple of the means that are presented in **Tables 3** and **4**. The data presented in this table have less rounding errors than those calculated from the means in **Tables 3** and **4**, since more significant figures were carried through the final calculation. [c] These mean and range values were obtained after totaling feed and water perchlorate intake at each dairy. These values cannot be calculated from the means in the rows above because with the exception of alfalfa hay only subsets of the products listed were fed at any one dairy. [d] Minimum. [e] Maximum.

to determine what influence, if any, the Colorado River had on the perchlorate concentrations of drinking water. The estimated perchlorate intake from drinking water ranged from 0.1 to 0.7 mg/cow per day and did not contribute significantly ($P > 0.84$) to the variation in total perchlorate intake across dairies. The perchlorate contribution from direct ingestion of water ranged from 0.2% to 20% total intake. In the one location where 20% of the total perchlorate was from water, the perchlorate level in the water was 4.1 μg/L perchlorate and total perchlorate intake was 3.4 mg/cow. This dairy was the exception and the perchlorate contribution from direct water consumption was generally less than 2% across dairies.

Assuming average Holstein and Jersey cow weights of 700 and 500 kg, respectively, the perchlorate doses received would range from 2.6 to 18.1 μg/kg per day. A previous study administered perchlorate doses up to 76.1 μg/kg per day and found no measurable health effects in dairy cattle (*24*). Comparison of perchlorate doses in cattle and humans requires adjustment for perchlorate metabolism that can occur during bovine ruminant digestion (*24*). If half of the bovine perchlorate intake is degraded during digestion, then the perchlorate doses we found in our study are approximately 100 times higher than typical human exposure (*1*) and approximately 10 times higher than the human RfD (*3*).

**Table 6.** Perchlorate, Iodide, Thiocyanate, and Nitrate Content of Milk Collected from 33 Dairies in the Southwestern United States

| | concn, μg/L | | | |
|---|---|---|---|---|
| | min[a] | max[b] | mean | median |
| perchlorate | 0.9 | 10.3 | 5.4 | 5.1 |
| iodide | 27 | 545 | 175 | 121 |
| thiocyanate | 89 | 2180 | 870 | 672 |
| nitrate | 398 | 8769 | 3144 | 2879 |

[a] Minimum. [b] Maximum.

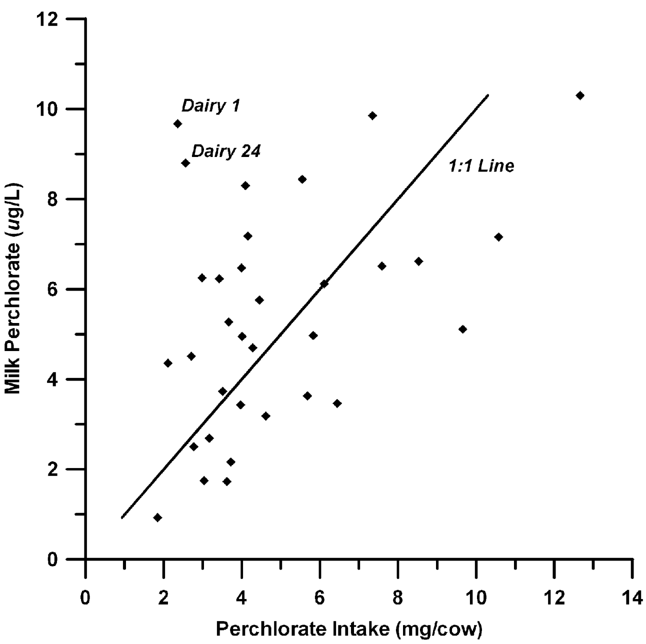


**Figure 2.** Perchlorate concentrations in milk by perchlorate intake as feed. Regression with or without outliers (dairys 1 and 24) is not significantly different than the 1:1 line shown.

**Perchlorate, Iodine, Thiocyanate, and Nitrate in Milk.** The perchlorate content of milk produced in the LCRR ranged from 0.9 to 10.4 μg/L, with an average of 5.4 μg/L (**Table 6**). These values are similar to levels reported for the United States by the U.S. Food and Drug Administration (FDA) (*17*) but slightly lower than those reported for Japan (*19*). It should be noted that out of 125 samples collected in the FDA survey, 63 samples were from Arizona and California and the results might be interpreted as being skewed toward an area influenced by the Colorado River. However, in the FDA survey the average concentrations of perchlorate within and outside the Arizona/California region were 5.5 and 6.0 μg/L, respectively.

For the study of Capuco et al. (*24*), the total perchlorate intake by dairy cows ranged from 0.50 to 40.5 mg per day, including feed, drinking water, and infused dose, and the resulting perchlorate content in milk ranged from 4.4 to 85.3 μg/L. In subsequent work (*43*) these data were used to report a strong relationship between perchlorate intake and the resulting milk concentration. Similarly, we found a statistically significant ($P < 0.05$) relationship between the perchlorate intake with feed and milk concentration, but with an $R^2$ value of 0.18 and a root-mean-square error estimate of 2.28 (**Figure 2**). Two observations (milk from dairies 1 and 24) appear to be outliers and were two of the three milk samples shipped to us on a later date because of the unavailability of milk at the dairy at the time of our sample trip. On the basis of this knowledge and subsequent joint parameter tests, the observations associated with dairies 1 and 24 were deemed to be statistical outliers (i.e., contaminated) and removed from the data set. A refit simple linear regression model using 31 dairies yielded a new *R* (*2*) value of 0.35 and a root-mean-square error estimate of 1.92. We also evaluated a zero-intercept regression model based on an expectation that a zero perchlorate intake load would result in a zero perchlorate milk concentration. When a zero-intercept model was refit to the reduced set of 31 dairy observations, the revised slope estimate (standard error) was computed to be 0.920 (0.069), which is not statistically different from 1 (F-score = 1.33, $p = 0.257$). This latter result suggests that the feed intake (mg/cow) to milk concentration (μg/L) exhibits a 1:1 relationship, under the zero-intercept modeling assumption, with or without the outliers. Our observed range of 1:1 to 1:2 [mg/cow]/[μg/L in milk] perchlorate ratio is considerably lower than the 1:10 to 1:8 ratio observed of Rice et al. (*43*). However, these authors (*43*) note that the constant ruminal infusion of perchlorate used in their experiment may not mimic feed and water ingestion patterns of commercial dairy cows.

We observed more variability than that observed by Rice et al. (*43*) where their data were derived from a small number of cattle, reared under closely controlled environmental conditions, and with a similar feed ration. In our evaluation we collected data from numerous dairies where the number of milk cows at each dairy ranged from less than 100 to almost 10 000, and where cows were subjected to a wide variation in feed rations and stress conditions. For example, some dairies used older shade structures and cooling facilities while others used modern and more effective shade structures and cooling facilities. We do not know to what extent variations in physiological stresses affected the perchlorate content of milk. In addition, we cannot rule out sampling error. Although we made every effort to collect a representative sample, the samples were relatively small composites collected from very large volumes of feed. Finally, we also do not know to what extent, if any, other chemicals used at dairies directly or indirectly influence the perchlorate variability of milk. Hypochlorites are widely used to sanitize milking equipment. In one study, hypochlorites were shown to have perchlorate concentrations ranging from 89 to 8000 μg/L (*44*). In a few samples that we collected from the hypochlorite canisters located at selected dairies, we found hypochlorite concentrations to range from 2 580 to 23 400 μg/L. Typically, the milking equipment is sanitized with very dilute solutions and subsequently rinsed with hypochlorite-free acidic solutions. The potential for perchlorate residues from hypochlorites to contaminate milk has not been evaluated. Another possible route of perchlorate exposure from hypochlorites is wash waste discharged into the waste lagoons. The contents of the waste lagoon are typically spread on nearby agricultural land, which is often used to produce feed and forage for the dairy. However, if this latter indirect source is an issue we would have incidentally accounted for it in our feed analysis.

We estimated milk recoveries by using mean summer production figures in Arizona of 31 and 25 kg/cow for Holstein and Jersey breeds, respectively. Using these numbers we estimated the approximate perchlorate recoveries in milk relative to intake in feed to range from 1.5% to 13.1% with a mean of 4.0%. We did not measure perchlorate in the urine and feces of dairy cows and we cannot perform a mass balance as performed in a previous study (*24*). Using mass balance the authors of this previous study (*24*) concluded that from 17% to 80% of the perchlorate is metabolized by the cows in the rumen.

Although the data for perchlorate concentration in milk followed a normal distribution, the data for thiocyanate were bimodal, and the data for iodide and nitrate were logarithmically distributed. Using the nonparametric Spearman rank order procedure we found no significant ($P > 0.1$) correlations among perchlorate, thiocyanate, nitrate, and iodide in milk. The iodide content of milk ranged from 26.6 to 545 $\mu$g/L. The mean milk iodide value of 175 $\mu$g/L that we observed is broadly consistent with milk iodide levels observed by others (*14, 22, 24*), and consistent with dairy milk as a significant source of iodine intake. The lack of correlation between perchlorate and iodide provides no evidence that these perchlorate doses competitively inhibit iodide transfer into milk. Further, we found no statistically significant ($P > 0.2$) relationship between perchlorate intake (mg/cow) and iodine content in milk. A previous study with radioiodide (*45*) has shown that bovine perchlorate intake in excess of 50 mg/day was required for a reduction of iodine in milk.

Another consideration concerning perchlorate exposure is the presence of other natural goitrogens in food, such as nitrate and thiocyanate (*46*). We found thiocyanate levels in milk to range from 89 to 2180 $\mu$g/L. Previous work has shown that thiocyanate levels in milk up to 8000 $\mu$g/L did not have any apparent adverse affect on thyroid function in humans (*47*). The nitrate content of milk in this study ranged from 390 to 8769 $\mu$g/L. The iodine uptake inhibiting potential of a mixture of NIS inhibitors can be estimated by using the perchlorate equivalent concentration (PEC). The PEC is defined as the sum of the concentrations of the inhibitors present divided by their NIS inhibition potency relative to perchlorate on a weight ingestion basis. A recent in vitro study with hamster ovary cells expressing human NIS has shown that the relative potency of perchlorate to inhibit iodide uptake at the NIS was 15 times that of thiocyanate and 240 times that of nitrate (*48*). Because thiocyanate has a serum half-life 18 to 29 times that of perchlorate and nitrate, it has been suggested that the relative potency of perchlorate on an ingested-weight basis would be 0.5 times that of thiocyanate. Because there are uncertainties as to how the in vitro study of Tonnachera et al. (*48*) can be extrapolated to humans, we used the more conservative estimates as follows $\text{PEC} = [\text{ClO}_4^-] + [\text{SCN}]/15 + [\text{I}^-]/30 + [\text{NO}_3^-]/240$. From this we estimate perchlorate as a percentage of total PEC ranged from 1% to 32% with a mean value of 10%. Additional work is needed to understand the interrelationships between iodide and anion inhibitors of iodide uptake. Unfortunately, we did not measure the iodide, thiocyanate, or nitrate contents of feed in this study and we cannot determine relationships between feed intake of these additional anions and milk composition.

**Summary.** In summary, results from this study show detectable perchlorate in all feed products used by the dairy industries of the southwestern United States. Variation in perchlorate intake across dairies could largely be explained by variation in the perchlorate intake from alfalfa product. However, perchlorate has been found at relatively high levels in alfalfa produced within and outside the lower Colorado River region. The perchlorate content in milk was significantly related to the perchlorate intake in feed, although the variation in milk could not be explained by feed intake alone. The perchlorate levels we observed in milk are similar to those observed nationally and we found no evidence that the use of feed products produced in the LCRR would significantly enhance perchlorate exposure through milk. Milk is both a source of iodine and a source of inhibitors of iodine uptake such as perchlorate, thiocyanate, and nitrate. A greater understanding the relative presence, potency, and toxicology of these competing anions is needed to fully assess the relative risks and benefits of milk consumption.


## ACKNOWLEDGMENT

We are grateful for the assistance of Mike Billotte of the United Dairymen of Arizona and Nyles Peterson of the University of California Cooperative Extension Service who assisted us in negotiating access to dairies and assisted the senior author with sample collection. We are also grateful to the dairymen in the region who allowed us access to their facilities for sample collection and provided us with the requested supporting informaton. We thank Dr. Purnendu Dasgupta for providing 3-chlorobenzenesulfonate. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.


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**Received for review November 20, 2007. Revised manuscript received March 14, 2008. Accepted March 20, 2008. Funding for this study was provided by a grant from the USDA National Integrated Food Safety Program.**

JF0733923