Lead in Calcium Supplements

Genine M. Scelfo and A. Russell Flegel

Environmental Toxicology, University of California, Santa Cruz, California, USA

Intercalibrated measurements of lead in calcium supplements indicate the importance of rigorous analytical techniques to accurately quantify contaminant exposures in complex matrices. Without such techniques, measurements of lead concentrations in calcium supplements may be either erroneously low, by as much as 50%, or below the detection limit needed for new public health criteria. In this study, we determined the lead content of 136 brands of supplements that were purchased in 1996. The calcium in the products was derived from natural sources (bonemeal, dolomite, or oyster shell) or was synthesized and/or refined (chelated and nonchelated calcium). The dried products were acid digested and analyzed for lead by high resolution-inductively coupled plasma-mass spectrometry. The method’s limit of quantitation averaged 0.06 µg/g, with a coefficient of variation of 1.7% and a 90–100% lead recovery of a bonemeal standard reference material. Two-thirds of those calcium supplements failed to meet the 1999 California criteria for acceptable lead levels (1.5 µg/daily dose of calcium) in consumer products. The nonchelated synthesized and/or refined calcium products, specifically antacids and infant formulas, had the lowest lead concentrations, ranging from nondetectable to 2.9 µg Pb/g calcium, and had the largest proportion of brands meeting the new criteria (85% of the antacids and 100% of the infant formulas). Key words: antacids, bonemeal, calcium supplements, dolomite, inductively coupled plasma-mass spectrometry (ICP-MS), infant formulas, lead, nutritional supplements, oyster shell, vitamins. Environ Health Perspect 108:309–313 (2000). [Online 21 February 2000]

http://ehpnet1.nihs.nih.gov/docs/2000/108p309-313scelfo/abstract.html

Most Americans (> 50% of all children under 5 and = 85% of teenage girls) do not ingest enough calcium (1,2) and many still ingest too much lead (3–5). Lead, in contrast to calcium, is not an essential nutrient, and it has no established toxicity threshold concentration (6). As a biochemical analog of calcium, lead interferes with calcium metabolism and many of its biologic functions (7–9). Recent studies indicate that low levels of lead exposure are correlated with irreversible fetal brain damage, hypertension, cardiovascular disease, kidney dysfunction, impaired bone synthesis, impaired sperm production, and osteoporosis (6). Because these diseases are in part attributed to perturbations of the calcium cycle by lead, the adverse effects of insufficient calcium and elevated lead intakes are additive (6).

Several studies indicate that lead absorption, bone lead remobilization, and lead toxicity are all reduced with a balanced diet and adequate calcium intake (6). These studies have often involved dietary calcium supplements because most foods have relatively low concentrations of calcium (7). However, some calcium supplements may also contain relatively high amounts of lead.

The primary “natural” sources of calcium for nutritional supplements (bonemeal, dolomite, and fossil oyster shells) all contain lead. Like calcium, lead is a naturally occurring element, and it is cycled through the biosphere as a calcium analogue (10). Although natural lead concentrations in calcium matrices are usually relatively low (e.g., < 0.5 µg/g dry weight), the concentrations may be markedly elevated by environmental and industrial lead contamination. Therefore, this paper describes a methodology for quantifying that contamination.

Background

The presence of lead in calcium supplements is of concern because some lead concentrations have been measured at toxic levels. These levels were initially detected in calcium supplements (bonemeal) in the 1960s (11) and precipitated a study and U.S. Food and Drug Administration warnings of potential lead contamination in calcium supplements (12,13). These initial concerns were substantiated in the 1980s by observations of the association of neurologic disorders in some patients who were taking either dolomite or bonemeal supplements and had relatively elevated lead concentrations in their hair (14). Those observations were further substantiated by a study in the early 1990s that also found relatively high, potentially toxic, levels of lead in some calcium supplements (15,16). More recently, lead contamination in a multivitamin was sufficient to confound a major evaluation of chelation therapy in children with blood lead concentrations > 20 µg/dL (17). Further investigation determined that the contaminated ingredient was ferrous fumarate (18).

Health concerns for the relatively high concentrations of lead in some calcium supplements, and any other ingested material, have increased with the results of recent lead toxicity studies. These studies failed to establish a discernible threshold for some measures of sublethal lead toxicity in humans (19). Consequently, the level of concern for childhood lead poisoning has recently been further lowered to blood lead concentrations of 10 µg/dL (20), and the adequacy of even that new standard has been questioned (10).

All of these concerns have led to the enactment of numerous state and federal measures both to reduce permissible levels of environmental and industrial lead exposure (6) and to advise the public of potential health risks associated with elevated lead exposures. In California, health advisory warnings must be put on the labels of consumer products with lead concentrations that amount to > 1.5 µg/daily dose (21). These values are based on a recommended adult daily dosage of 1 g calcium. [The recommended daily dosage of calcium for children 1–3 and 4–8 years of age is 500 and 800 mg, respectively; recommendations for older adults range from 1,000 to 1,300 mg calcium (22).] Consequently, relatively sophisticated analytical techniques are now required to accurately determine whether such advisories are appropriate and needed for different consumer products, including calcium supplements.

Methods

Samples. One hundred thirty-six brands of nutritional supplements containing calcium were purchased in 1996 from commercial outlets in California. The nutritional supplements included calcium supplements (83 brands), vitamin–mineral supplements (27 brands), antacids (20 brands), and infant formulas (6 brands). To determine whether the lead concentrations of those diverse samples were representative of the different products and types of calcium supplements, we measured interlot variations for 28 brands. We analyzed two to nine (average n = 6) lot samples of those products.

Address correspondence to G. Scelfo, Environmental Toxicology, Applied Sciences 269, University of California, Santa Cruz, CA 95064 USA. Telephone: (831) 459-3563. Fax: (831) 459-2088. E-mail: gscelfo@es.ucsc.edu.

We thank S. Hibdon and R. Franks for analytical support.

This research was partially supported by funds from the California Attorney General's Office; the Natural Resources Defense Council, and the University of California Toxic Substances Research and Teaching Program.

Received 1 July 1999: accepted 26 October 1999.
The calcium used in the supplements was either from a natural source or was synthesized and/or refined (as specified on the product label). The advertised natural calcium sources were bone meal (hydroxyapatite or calcium phosphate (CaPO₄)), dolomite (CaMg(CO₃)₂), and fossil oyster shell (CaCO₃). There were two major types of synthesized and/or refined sources of calcium: calcium salts and calcium bound with various organic chelates. Calcium carbonate (CaCO₃) was the most commonly reported calcium salt. Other synthesized products included calcium phosphate, calcium sulfate, and calcium chloride. Many of the chelated-calcium products contained one or more of the following chelates: citrate, gluconate, aspartate, ascorbate, stearate, malate, fumarate, and lysinate. Calcium citrate was the most common of those chelates.

The vitamin–mineral and calcium supplements included both natural source calcium (bone meal, dolomite, and fossil oyster shell) and synthesized and/or refined calcium (chelated and nonchelated). We analyzed 26 oyster shell brands, 9 bone meal brands, and 5 dolomite brands (natural source supplements). We analyzed 33 chelated and 37 nonchelated brands (synthesized and/or refined calcium supplements).

**Sample processing.** The samples were digested in Teflon labware that had been cleaned in a high-efficiency particulate air (HEPA) filtered, trace-metal-clean laboratory to minimize contamination (19). This protocol involved sequentially cleaning the labware in a series of baths in solutions (1 week each) and rinses (five per solution) in a three-step order: (a) we used a detergent solution (Micro; American Scientific Products; McGraw Park, IL) and deionized water rinses; (b) we used a 6-N HCl (reagent grade) solution and ultrapure (Milli-Q; Millepore Corp., Bedford, MA; 18 MQ/cm) water rinses; and (c) we used a 7.5-N HNO₃ (trace metal grade) solution and ultrapure water rinses. The labware was then air dried in a polypropylene laminar airflow-exhausting hood.

After we determined the mean product weight of 5–10 sample units (e.g., tablets or capsules) or a 15-mL solution within each lot, we placed aliquots of sample homogenates (0.25–0.5 g powder or 10–15 mL liquid) in the containers. The weighed product units were ground into a fine powder using an acid-cleaned mortar and pestle. Sample aliquots were placed in acid-cleaned Teflon screw-cap vials, dried overnight in an oven at 75°C, cooled to the ambient temperature, and then weighed.

The dried samples were dissolved in 10 mL concentrated HNO₃ (trace metal grade). When necessary, some sample vials were placed in an ice bath and the acid was slowly and incrementally added to control rapid oxidation (e.g., foaming) of the samples. The dissolved solutions were refluxed in the capped vials on a ceramic hot plate (modified for trace metal analyses) for 4 hr at a low (≤ 50°C) temperature to prevent excessive foaming. The dissolved solutions were then refluxed at a higher temperature (> 85°C) until the solution was transparent, as specified by the National Food Library (NFL) (22). Finally, the samples were evaporated to dryness and reconstituted in 10-mL 1 N HNO₃ (trace metal grade) for elemental analyses.

However, that digestion was insufficient for lead concentration measurements by graphite furnace atomic absorption spectrometry (GFAAS). We determined that to accurately analyze by GFAAS, the digested sample solutions must be both transparent and colorless, indicating the total breakdown of the organometal complexes. This additional requirement substantially increased the time for the digestion step from 1–2 days to 7–10 days, based on a series of analyses in our laboratory and corroborated by the NFL (22).

Conversely, the initial digestion to a merely transparent solution proved sufficient for lead concentration measurements by high resolution inductively coupled plasma mass spectrometry (ICP-MS). This method involves atomizing the solution in a plasma with a temperature of approximately 7,000 K. The high temperature is sufficient to destroy any organometal complexes before elemental analyses, as indicated by the quantitative recovery (96.3 ± 4.8%) of lead in a bone meal standard reference material [SRM 1486; National Institute of Standards and Technology (NIST), Gaithersburg, MD] by ICP-MS as compared to the relatively low (51.0 ± 5.6%) recovery by GFAAS.

**Instrumental analyses.** As previously indicated, the initial attempts to measure lead concentrations in the sample solutions were made by GFAAS, using a SIMAA 6000 (Perkin-Elmer, Norwalk, CT) with Zeeman background correction. The instrument was initially calibrated with replicate analyses (n = 7) of a Perkin-Elmer certified mixed element standard, with lead recovery of 98.9 ± 1.7%. To control for matrix effects, samples were measured using a matrix modifier [NH₄H₂PO₄ + Mg(NO₃)₂]. Although concentrations were calculated using the method of standard additions, this still did not appear to correct for all matrix effects.

Based on problems incurred with the initial GFAAS analyses, subsequent measurements were made by ICP-MS. These measurements proved more accurate and efficient for measuring lead in calcium supplements. The analyses were made with a Finnegan MAT Element high-resolution magnetic sector inductively coupled plasma-mass spectrometer (Finnegan, Bremen, Germany). To maintain high quality instrument performance, solutions for ICP-MS analysis did not exceed 0.1% total dissolved solids. These digest solutions were made with 25- to 50-fold dilutions with a 1-µg/L (ppb) bismuth internal standard solution in 1 N HNO₃ (i.e., 100–250 µL digest was added to 5 mL 1 µg/L bismuth solution in trace-metal-clean polypropylene vials). The calibration standards (0, 0.5, 1.0, 2.5, and 5.0 µg/L) were also spiked with 1 µg/L bismuth.

We derived the lead concentration analyses from instrumental scans of three lead isotopes (206Pb, 207Pb, and 208Pb) and bismuth (209Bi). The sum of intensities for the stable lead isotopes was normalized to the bismuth internal standard to correct instrumental variations in sensitivity. Spiking the samples with 209Bi precluded the analysis of supplements containing large amounts of bismuth, but an alternative internal standard such as thallium could be used for analyses of that type of supplement.

**Quality assurance.** We used quality assurance samples from each sample batch to assess the precision and accuracy of analyses within our laboratory. Ten percent of the samples for each batch were digested and analyzed in duplicate to determine the precision of the digestion, and we used replicate instrument measurements to determine instrument precision. We assessed replicate dilutions of the digest solutions for ICP-MS analyses to determine the precision of pipetting the sample digests. We analyzed three solutions of a standard reference material (NIST SRM 1486 bone meal) concurrently with each sample batch to quantify the accuracy of the measurements. Three procedural blanks were analyzed concurrently with each sample batch to quantify the sample contamination associated with processing and the detection limit.

We also used quality assurance samples to compare the precision and accuracy of analyses between laboratories (Table 1). A highly refined CaCO₃ product (Specialty Minerals, Inc., Adams, MA) was analyzed by four laboratories. The laboratories included the manufacturer’s laboratory, two independent laboratories contracted by the manufacturer, and our laboratory at the University of California Santa Cruz (UCSC). All of the laboratories measured lead concentrations in the CaCO₃ product using three instruments: GFAAS, quadrupole ICP-MS, and high resolution magnetic sector ICP-MS, respectively.

Because the sample matrices for most calcium supplements are much more complex than those of refined calcium supplements, we also conducted a second interlaboratory calibration with the NFL in
Dublin, California (Table 2). This intercalibration included analyses of seven brands of calcium supplements, with one or two samples from each of the five types of calcium supplements. Twenty tablets of each product were ground, homogenized, and dried using trace-metal-clean techniques. At the NFL, the sample aliquots were acid digested and analyzed by GFAAS, with Zeeman effect background correction and using the standard additions method with a matrix modifier [PD + Mg(NO$_3$)$_2$]. At UCSC, the sample aliquots were processed and measured by high resolution ICP-MS, as detailed in this paper.

**Quality assurance comparisons with recent measurements of lead in calcium supplements.** Current levels of sensitivity [method detection limits (MDLs) and limit of quantitation] and precision [coefficient of variation (CV)] in measurements of lead in calcium supplements are listed in Table 3, which summarizes reported analytical parameters for the measurements using different instrumentation (23-25). These instruments include anodic stripping voltametry (ASV), flame and graphite atomic absorption spectrometry (FAAS and GFAAS, respectively), and quadrupole ICP-MS. We also used high-resolution magnetic sector ICP-MS; those measurements are detailed in this paper.

**Results and Discussion**

Two-thirds of the 136 products purchased in 1996 failed to meet the 1999 California criteria for acceptable lead levels in consumer products. Although there is no statistically significant difference ($p < 0.05$, analysis of variance) between the supplement types measured in this study, antacids had the lowest average lead concentration with the least variation (Table 4). We did not include the infant formulas (all < MDLs) in the statistical test.

**Lead concentrations in synthesized and refined calcium supplements.** Figure 1 illustrates the distribution of lead in supplements made with synthesized calcium. Three nonchelated brands and six chelated brands exceeded the federal limit of 7.5 μg lead/g calcium (or 6 μg lead/800 mg calcium) (Figure 1). Although approximately three-fourths of the nonchelated brands ($n = 28$) and almost two-thirds of the chelated brands ($n = 21$) met the July 1997 California limit of 4 μg lead/g calcium (or 3.2 μg lead/800 mg calcium), only a small proportion of these (10 nonchelated and 3 chelated) would meet the new April 1999 California limit (21) of 1.5 μg lead/g calcium (or 1.2 μg lead/800 mg calcium).

Generally, the lowest lead levels in all of the materials that we analyzed were found in the infant formulas and the antacids, which all contained either synthesized and/or refined calcium. Lead concentrations were nondetectable (< 0.02 μg/g) in all infant formulas tested, and all brands of infant formulas and antacids were in compliance with both the federal and the California limits for lead that were in effect at the time of analysis. However, three brands of antacids, based on these analyses, would now be in noncompliance with the new (April 1999) standards in California (Figure 1) (27).

**Lead concentrations in natural calcium supplements.** Figure 2 shows the distribution of lead concentrations in the natural source calcium products. Two dolomite brands and one oyster shell brand exceeded the federal limit (Figure 2). Two of five dolomite brands met the California July 1997 limit, and none of these would meet the lower April 1999 limit. Nineteen of 26 oyster shell brands met the California July 1997 limit, only 5 of which would meet the lower April 1999 limit. All nine bonemeal brands met the federal limit; however, although eight brands met the California July 1997 limit, only half of these would meet the California April 1999 limit.

**Table 1. Lead concentration (μg/g) measurements for a highly refined calcium carbonate powder for four laboratories using three instruments.**

| Laboratory  | Instrument | Lead concentration (μg/g dry weight) | Mean ± SD | No. |
|-------------|------------|-------------------------------------|----------|-----|
| Manufacturer | GFAAS      | 0.187 ± 0.006                       | 13       |
| Independent A | GFAAS       | 0.184 ± 0.005                       | 3        |
| Independent B | Quadropole ICP-MS | 0.177 ± NA                           | 1        |
| UCSC        | High resolution ICP-MS | 0.167 ± 0.004                      | 3        |

UCSC, University of California Santa Cruz. Interlaboratory calibration: lead analysis of refined CaCO$_3$ powder (lot no. A-3-070-26; Specialty Minerals, Inc., Adams, MA).

**Table 2. Interlaboratory calibration: lead analysis of calcium supplements.**

| Sample type | Sample code | NFL (GFAAS) | UCSC (ICP-MS) | RPDP |
|-------------|-------------|-------------|---------------|------|
| Refined, lozenge | 216-ref-z | 0.06 | 0.06 | 2.4 |
| Oyster, tablet | 161-ov-t | 1.07 | 1.21 | 12 |
| Oyster, tablet | 94-ov-t | 1.74 | 1.53 | 13 |
| Refined, capsule | 206-ref-c | 0.99 | 0.83 | 17 |
| Chelated, tablet | 209-che-t | 1.42 | 1.18 | 19 |
| Bonemeal, capsule | 220-bon-c | 0.48 | 0.38 | 24 |
| Dolomite, tablet | 139-dol-t | 2.60 | 1.72 | 41 |

RPDP, relative percent difference. Measured lead concentration (μg/g dry weight).

**Table 3. Comparison of quality assurance parameters with previously reported analytical lead measurements in calcium supplements.**

| Instrument | MDL (μg/g) | LOQ (μg/g) | CV (%) | Recovery (%) |
|------------|------------|------------|--------|--------------|
| Anodic stripping voltametry (ASV) | 0.42$^a$ | 1.4 | 18 | 92 |
| FAAS | 0.50$^b$ | 1.7 | 29 | 147 |
| GFAAS | 0.12$^c$ | 0.40 | 7 | 100 |
| ICP-MS (quadropole) | 0.02$^d$ | 0.03 | 9 | 89 |
| ICP-MS (magnetic sector) | 0.02$^e$ | 0.06 | 4.9 | 90-120 |

Data are from the analysis of standard reference materials with a matrix comparable to calcium supplements (e.g., NIST SRM 1466 bonemeal).

$^a$Parameters include MDL, LOQ, and CV. $^b$Instruments include ASV, FAAS and GFAAS, and ICP-MS.$^c$Bourgojn et al. (19). $^d$Sittenon and Thompson (29). $^e$West Coast Analytical Service (26). This paper.
Table 4. Reported lead concentrations (µg/g dry weight) in various calcium supplements by several investigators over a 20-year period.

| Reference             | Bonemeal | Dolomite | Oyster shell | Refined | Refined antacids | Chelated | Infant formulas (powder) | Infant formulas (canned liquid) |
|-----------------------|----------|----------|--------------|---------|------------------|----------|--------------------------|-------------------------------|
| Crosby 1977 (11)      | 190      |          |              |         |                  |          |                          |                               |
|                       | n = 1    |          |              |         |                  |          |                          |                               |
| Capar and Gould 1979 (12) | 4.16 ± 1.78 |          |              |         |                  |          |                          |                               |
|                       | n = 20   |          |              |         |                  |          |                          |                               |
| Roberts 1983 (14)     | 9.88 ± 3.2 | 5.64 ± 1.99 |          | 0.30 ± 0.35 | (0.08-0.7)          |          |                          |                               |
|                       | n = 8    | n = 8    |              | n = 3    |                  |          |                          |                               |
| Dabeka and McKenzie 1987 (27) |          |          |              |         |                  |          |                          |                               |
| Bourgoin et al. 1993 (29) | 2.67 ± 2.74 | 1.11 ± 0.71 | 2.11 ± 1.33 | 0.34 ± 0.24 | -              |          |                          |                               |
|                       | n = 6    | n = 9    |              | n = 25   |                  |          |                          |                               |
| Sittenen and Thompson 1994 (28) | 4.27 ± 2.71 | 0.94 ± 0.51 | 0.67 ± 0.54 | -          |                  |          |                          |                               |
|                       | n = 3    |          |              | n = 17   |                  |          |                          |                               |
| This paper (brands purchased in 1996) | 0.60 ± 0.39 | 0.97 ± 0.49 | 0.88 ± 0.51 | 0.73 ± 1.60 | 0.12 ± 0.16 | 0.57 ± 0.54 | <0.02                  | <0.02                          |
|                       | n = 9    | n = 5    |              | n = 26   | n = 37            | n = 20   | n = 3                    | n = 3                          |

n, number of brands analyzed. Values are mean ± SD (range).

*Collected in 1980. *Lead-soldered cans. *Collected in 1985. *Lead-free cans. *Collected in 1996.

Some of the historical reports of measurements of lead in nutritional supplements are listed in Tables 3 and 4. Table 3 shows the marked improvement in the precision (CV) in lead analyses over the past 20 years. This is notable because previous levels of precision are insufficient for measurements to meet the new advisory requirements for lead exposure (i.e., 1.5 µg lead/dose).

**Temporal variations of lead in calcium supplements.** The data in Table 4 provide some perspective on historic and current variations in lead concentrations in calcium supplements over the past 20 or more years (11,12,14,16,25-27). As previously indicated, concerns with lead contamination in nutritional supplements were catalyzed by reports of remarkably elevated lead levels (20-532 µg/g) in dolomite, bonemeal, and infant formulas (11,14,27) and relatively high levels (4.88 µg/g) in oyster shell supplements (16). The mean lead concentrations for each corresponding supplement type purchased in 1996 were lower than the first published values. There is a statistically significant decrease (p < 0.05, t-test) in lead concentrations in some types of supplements (e.g., bonemeal, oyster shells, and infant formulas), but not in others (e.g., dolomite). There also appear to have been relatively high variations in lead concentrations in refined and chelated calcium supplements throughout that period.

The apparent temporal decrease in lead concentrations in bonemeal, oyster shells, and infant formulas is tentatively attributed to corresponding reductions in lead contamination and improvements in processing those materials. Environmental exposures to industrial lead have decreased by orders of magnitude after the systematic elimination of leaded gasoline emissions in much of North America and Europe over the past 20 years (28). Food and nutritional products, such as infant formulas, are mostly stored in lead-free cans instead of lead-soldered ones. Many manufacturers that used bonemeal for calcium supplements now use the bones of younger bovines or equines, which have less accumulation of industrial lead. And, most recently, many manufacturers have shifted to using only red bone marrow because it contains less lead and is less subject to lead contamination than outer bone. Similarly, many manufacturers that use fossil oyster shells for calcium supplements have been using older deposits that contain less lead. Those temporal reductions contrast with the relative consistency in lead concentrations in dolomite supplements over the past 20 years, which may be due to the relative homogeneity of lead concentrations in those deposits (15).

**Summary**

These data are consistent with previous measurements of lead in calcium supplements. They indicate that some contain relatively low concentrations of lead (< 0.5 µg/g dry weight), whereas others contain relatively high concentrations. Some of the latter concentrations exceed the most recent criteria established to limit lead exposure in California (> 1.5 µg/g).

The temporal decrease of lead concentrations in some calcium supplements, indicated by previous analyses, is substantiated by this study. This includes decreases in lead concentrations in products derived from bonemeal and oyster shells, but not those derived from dolomite, over the past 20 years.
years. In addition, the data corroborate previous reports of the relatively high variations in lead concentrations in refined and chelated calcium supplements during that period. These new data demonstrate that relatively low concentrations (< 0.5 µg/g dry weight) may now be accurately measured in complex calcium matrices. These matrices require the adaptation of trace-metal-clean techniques to minimize contamination during sampling, processing, and analyses. The matrices also require the adaptation of rigorous quality assurance protocols to preclude erroneous measurements, which will tend to underestimate lead concentrations in calcium supplements.

As previously noted, these reported concentrations are for a limited number of analyses of the brands that we collected in 1996. The analyses were conducted before the settlement of litigation on the disclosure of lead concentrations in calcium supplements in California. The disclosure of lead concentrations in calcium supplements catalyzed efforts to further reduce lead concentrations in those supplements. Consequently, lead concentrations in some of those products may now be lower than the values in this paper.

In addition, the exposure risk from lead in calcium supplements may be relatively small, even though the contribution of lead from a daily dose of those supplements (median = 2.38 µg) to the average total daily dietary intake (5–11 µg/day) may be relatively large (29). This disparity is due to the much higher supplemental concentrations of calcium, which decrease gastrointestinal lead absorption (30). Several studies of factors that influence the dietary assimilation of lead show this decrease in lead absorption (31).

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