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Declining Blood Lead and Zinc Protoporphyrin levels in Ecuadorian Andean Children

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Abstract

Objectives—To investigate current lead (Pb) exposure in children living in Andean Ecuadorian communities. Blood Pb (PbB) and zinc protoporphyrin (ZPP) levels were used respectively as biomarkers of acute and chronic Pb poisoning. The current PbB-ZPP levels were compared with previous pediatric PbB-ZPP levels recorded over years in the study area.

Design and Methods—Samples of whole blood were collected from 22 Andean children of Quechua and Mestizo backgrounds and measured for PbB concentrations by graphite furnace atomic absorption spectroscopy. ZPP/heme ratio and ZPP whole blood (ZPP WB) levels were measured with a hematofluorometer.

Results—The mean PbB level for children in the current study group was 14.5 µg/dL, which was significantly lower than the mean PbB level of 41.1 µg/dL found in the same study area in the 1996–2000 test period, and lower than the 22.2 µg/dL mean level found in the 2003–2007 period. The current mean ZPP/heme ratio was 102.1 µmol/mol, and the mean ZPP WB level was 46.3 µg/dL, both lower than values previously found in children in the study area.

Conclusion—While the current pediatric PbB-ZPP levels in the study area remain elevated in some children, the overall levels indicate a decline relative to levels observed in the same Pb-

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contaminated area in the period between 1996 and 2007. The elevated ZPP levels suggest a history of chronic Pb exposure, and potential iron deficiency in some children. The overall reduction in PbB-ZPP levels suggests a positive outcome of a Pb-exposure education and prevention program, and the therapeutic intervention of succimer chelation therapy.

Keywords

Children; Blood; Lead; Zinc Protoporphyrin; Andes

Introduction

Pediatric lead (Pb) poisoning continues to be a global health burden, particularly in developing countries where Pb is used in occupational activities (such as, Pb smelting, battery recycling, and Pb-glazing of ceramics) in which children participate directly, or are exposed from living in close proximity to the Pb-contaminated sites [1–6]. Pb is highly neurotoxic, with deleterious effects on the nervous system, particularly the developing nervous system. Pediatric Pb exposure, even at low exposure levels has been associated with neurocognitive impairment, including adverse effects on intellectual performance [7–9]. The main route of Pb poisoning in children is via pica or the ingestion of Pb-contaminated substances. In children, approximately 40–50% of ingested Pb is absorbed through the gastrointestinal tract and distributed to the soft tissues, including brain, liver, and kidneys, and more than 70% of absorbed Pb is stored in bone and teeth [10].

The conventional and most reliable biomarker for acute or recent pediatric Pb exposure is the concentration of Pb in whole blood (PbB). One of the targets of Pb poisoning in children is the hematologic system, where Pb inhibits the activities of enzymes responsible for heme biosynthesis [10]. Following exposure, Pb in blood is concentrated primarily in erythrocytes, where it binds to delta-aminolevulinic acid dehydratase (ALAD) [10–12]. Pb inhibits the enzymes ALAD and ferrochelatase, which is necessary for the chelation of iron (Fe) by protoporphyrin [10–12]. The resulting accumulation of protoporphyrin in the absence of Fe attracts zinc as a replacement, forming zinc protoporphyrin (ZPP). In cases of prolonged or chronic Pb exposure, Fe in hemoglobin (Hb) is essentially replaced by zinc. Elevated ZPP levels indicate Pb-induced inhibition of heme biosynthesis [10]. Whereas, PbB level is a measure of recent exposure, the ZPP/heme ratio may be useful as a biomarker for prolonged Pb exposure, since elevated ZPP levels lag elevated PbB levels by weeks to months, and may reflect chronic Pb exposure for up to two years [13–16]. Elevated ZPP, a biomarker of Pb toxicity, may also be an indication of Fe deficiency in children, although this is not invariably the case, since the ZPP measure has been shown to have a high false-positive rate for indication of Fe deficiency [16].

Children living in rural Andean communities of Ecuador where Pb glazing of ceramics is a local backyard industry have been found to have high PbB and ZPP levels [17–21]. These elevated PbB levels have been significantly associated with abnormal neurocognitive performance in the children living in the study area [22–24]. In addition, elevated levels of Pb found in the milk of breast-feeding mothers and in the blood of mother-infant pairs living in the same study area suggest that some children are already exposed to Pb during the prenatal and breast-feeding periods [25].

Following our initial investigations, a Pb-exposure education and prevention program was initiated in the study area, and subsequently, intervention with succimer (DMSA) medical treatment was provided for children living in the study area who were found to have elevated PbB levels [18, 26]. Case studies of children in the study area have shown improvement in neurocognitive performance of some children as the PbB levels declined

[27]. As part of our ongoing Pb-exposure education and prevention efforts, we have continued to monitor PbB levels in the study area. The purpose of the present study was to further investigate the Pb exposure levels in a cohort of children currently living in the study area, and to compare the findings with earlier PbB levels obtained on cohorts of children living in the same communities between 1996 and 2007.

Materials and methods

Participants and location

In 2012, 61 inhabitants of the Pb-contaminated study area, of which 22 were children, were examined for PbB and ZPP levels. The present study focuses exclusively on the 22 children living in the study area. The PbB-ZPP levels for the 39 adults are presented elsewhere. The participants consisted of 12 females and 10 males ranging in age from 1.5 years to 16 years who were available for testing. The mean age for the current group of children was 9.3 years (SD: 4.1; median: 9.5) living in villages around La Victoria in the Cotopaxi region of Ecuador at an altitude of approximately 2,850 m. In addition to La Victoria Centro, the children who were tested resided in the communities of La Victoria, including El Tejar, Mulinlivi and El Paraiso. The source of Pb exposure in the study area is discarded Pb-acid automobile storage batteries from which adults and children extract Pb for use in the glazing of ceramics, particularly roof tiles produced in a local Pb glazing cottage industry. Hand-to-mouth ingestion of Pb-contaminated food, dust and soil, and the inhalation of small air-borne particulates from the heavily Pb-laden smoke produced by the glazing kilns are the primary routes of Pb exposure in the children [18,19]. All participants were examined at the local Subcentro de Salud in La Victoria, Ecuador.

Informed consent was obtained from the parents/guardians of the children prior to testing. This study was approved by the Human Studies Committee (Comité de Bioética) of the Universidad San Francisco de Quito. The study was conducted under the auspices of Universidad San Francisco de Quito Colegio Ciencias de la Salud, Escuela de Medicina in Quito, Ecuador. The results of this investigation were presented to the parents/guardians of the children who participated in the study. The participants and their families were counseled regarding their Pb exposure, and were referred to local health officials for medical intervention where appropriate.

Blood tests

Samples of 2–4 mL of whole blood were drawn by venipuncture from the participants following thorough cleaning of the skin using swabs containing isopropanol. The samples were collected from the antecubital vein and kept in 4-mL Vacutainer collection tubes with Li-heparin. All whole blood samples were stored in a refrigerated container. The blood samples were later analyzed for Pb concentration by graphite furnace atomic absorption spectroscopy with Zeeman background correction (Perkins Elmer 5000 Zeeman HGA-500 spectrophotometer, Norwalk, Connecticut). ZPP/heme was measured directly using a hematofluorometer (ProtoFluor-z, Helena Laboratories, Inc., Beaumont, TX), which presents results in $\mu\text{mol/mol}$ heme. Control samples (Kaulson Laboratories, West Caldwell, NJ) were run at low, medium and high levels. ZPP WB values are expressed in $\mu\text{g/dL}$ with an assumed hematocrit of 35%. The normal reference ranges used were 30–70 $\mu\text{mol ZPP/mol heme}$, and 15–36 $\mu\text{g ZPP/dL whole blood}$. All blood tests were performed at the Boston Children's Hospital Department of Laboratory Medicine.

Statistical analysis

The mean, standard deviation, range, and median were calculated for PbB concentration, ZPP/heme ratio and ZPP WB level obtained on each participant in the study. Because some

of the PbB and ZPP data were skewed, nonparametric statistics were used in the data analysis. Differences between means were analyzed using the Mann-Whitney *U* test. The association between PbB level and ZPP/heme ratio was analyzed by Spearman *rho* correlation analysis. All *Z* and *p* values reported for the Mann-Whitney *U* test and the Spearman correlation coefficient are tied values. An alpha level of .05 was accepted as an indication of statistical significance.

Results

To determine the Pb exposure levels over time, the results for the children in the current study were compared to Pb exposure data pooled over 5-year intervals from cohorts of children tested previously in the same Pb-contaminated study area. The aggregate data are illustrated in the box plots of Figure 1, which show the distribution of PbB levels of the children in the study area for three different cohorts tested in 5-year intervals: 1996–2000 (*n* = 274), 2003–2007 (*n* = 329), and 2012 (*n* = 22). The mean PbB level obtained for the children during the 2012 test period (current study) was 14.5 µg/dL (SD: 7.9; median: 14.0; range: 4.0–37.0), and significantly lower than the mean PbB level of 41.1 µg/dL (SD: 24.6; median: 37.6; range: 6.1–128.2) found in the same study area in the 1996–2000 test period (Mann-Whitney *U*: *Z* = −5.570, *p* = < .0001). The mean PbB level of 14.5 µg/dL for the current participants was also lower than the mean PbB level of the children tested in the 2003–2007 examination period (mean: 22.2 µg/dL; SD: 19.8; median: 15.0; range: 2.1–107.0), but the difference did not reach statistical significance (Mann-Whitney *U*: *Z* = −1.056, *p* = .291). Overall, the data presented in Figure 1 illustrate group reductions in PbB levels over time, indicating declining Pb exposure in the study area.

The Pb exposure levels of the children in the study area were further probed by comparing the PbB levels at yearly intervals to examine more closely the decline in Pb exposure. Figure 2 shows the PbB levels by year of cohorts of children tested in the study area from 1996 to the current group tested in 2012. Similar to the box plots of Figure 1, the line graph shown in Figure 2 illustrates an overall decline in PbB levels over the years, beginning in 1996. The mean PbB level of 14.5 µg/dL obtained in the current study was significantly lower than the mean PbB level of 44.6 µg/dL found in the 1996 cohort (Mann-Whitney *U*: *Z* = −5.061, *p* = < .0001). An exception to this declining trend was the mean PbB level of 45.8 µg/dL for a group of children tested in 1999 (Figure 2) that indicated an elevation rather than a reduction in PbB levels, suggesting a period of increased Pb exposure. The decline in PbB levels by year was further explored by examining the percentage of children with PbB levels ≤ 5 µg/dL, 10 µg/dL, 45 µg/dL, and 70 µg/dL. The 5 µg/dL level was chosen because it is the current reference value used in the United States; the 10 µg/dL level was until recently the Centers for Disease Control and Prevention's (CDC) action level; 45 µg/dL is the CDC's recommended level for chelation therapy; and the 70 µg/dL level is considered a medical emergency by the CDC. The percentage of children in each of the aforementioned categories are displayed in Table 1, which shows that most of the children had PbB levels above the 5 and 10 µg/dL categories for each year from 1996 to 2012. However, after 2000, the percentage of children with PbB levels ≤ 10 µg/dL declined by as much as 39.4%. In addition, it can be seen in Table 1 that none of the children tested in 2012 had PbB levels ≥ 45 µg/dL.

The PbB levels of the children were further analyzed by separating the children into younger (0–10 years) and older (11–16 years) age groups. The age group results are displayed in Figure 3A, which shows a greater peak in the Pb exposure level for the older age group for the 1999 cohort. Statistical analysis using the Mann-Whitney *U* test revealed that in 1999, the older age group had a mean PbB level of 56.2 µg/dL, which was significantly higher than the mean PbB concentration of 40.5 µg/dL for the younger age group (*Z* = −3.027, *p* = .

002). In 2000, the older age group, with a mean PbB level of 45.7 µg/dL, was also significantly different from the younger age group, who showed a mean PbB level of 29.7 µg/dL (Mann-Whitney *U*: $Z = -2.483$, $p = .013$). For the years 1996, 1997, 1998, 2003, 2006, 2007 and 2012, there were no significant differences between the older and younger age groups.

To determine if the decline in PbB levels showed the same time course pattern for males and females, the timeline data from 1996 to 2012 ($n = 625$) were further analyzed for males and females separately, and are shown in the line graph of Figure 3B. It can be seen in Figure 3B that the males and females showed similar declining PbB concentration curves from 1996 to 2012, with the females tending to have lower PbB levels. However, Mann-Whitney analyses for each year revealed that none of the differences in PbB levels between the females and males reached statistical significance. To guard against a type II or beta error, gender differences were further analyzed by pooling all of the PbB data from 1996 to 2012 ($n = 307$ males, 318 females). A Mann-Whitney *U* analysis on the larger sample indicated a significant difference for gender, showing the males to have significantly higher PbB levels than the females ($Z = -2.804$, $p = .005$). It can further be observed in Figure 3B that the female children had an upswing in mean PbB level in 1999, similar to that of the males. However, in 1999, 52.2% of the male participants had PbB levels ≥ 45 µg/dL, whereas, only 30.0% of the female participants had PbB levels ≥ 45 µg/dL.

The mean ZPP/heme ratio for the current group of children was 102.1 µmol/mol (SD: 75.3; median: 64.0; range: 44.0–281.0), and the mean ZPP WB level was 46.3 µg/dL (SD: 34.1; median: 29.0; range: 20.0–127.0). Spearman *rho* correlation coefficient showed a significant correlation of 0.597 ($Z = 2.738$, $p = .006$) between ZPP/heme and PbB levels for the 2012 group. Correspondingly, there was a significant correlation between ZPP WB and PbB (*rho* = 0.591; $Z = 2.709$; $p = .006$). Because ZPP/heme correlates better with PbB levels above 25 µg/dL, a second correlation analysis was performed in which 7 of the 22 children with PbB ≤ 10 µg/dL were temporarily removed from the data analysis. The resulting Spearman *rho* correlation coefficient between PbB and ZPP/heme for the children with PbB levels > 10 µg/dL was 0.865 ($Z = 3.237$, $p = .001$).

Figure 4 tracks the PbB concentration (A) and ZPP/heme levels (B) obtained for three years (2006, 2007 and 2012) on 261 Pb-exposed children living in the study area. For this sample of 261 children, a Spearman *rho* correlation analysis showed a statistically significant and high correlation between the PbB level and the ZPP/heme ratio averaged across the three years (*rho* = 0.719; $Z = 11.606$; $p < .0001$). The association of ZPP levels with PbB concentration can be seen clearly in the line graphs of Figure 4, which show declining ZPP levels that parallel the course of the decline in PbB concentration. This relationship was further quantified by calculations indicating that from 2006 to 2007, the PbB levels declined by a factor of 1.45 (Mann-Whitney *U*: $Z = -3.092$, $p = .002$) and the ZPP levels declined by a similar factor of 1.35 (Mann-Whitney *U*: $Z = -1.287$, $p = .198$). Between 2007 and 2012, the PbB levels declined by a factor of 1.26 (Mann-Whitney *U*: $Z = -.059$, $p = .953$), and the ZPP levels declined by a comparable factor of 1.13 (Mann-Whitney *U*: $Z = -.363$, $p = .716$). The decline in Pb exposure is also affirmed by the percentage of children whose ZPP/heme ratios were above the normal range of 30–70 µmol/mol. In 2006 and 2007, 54.0% and 47.9%, respectively, of the children had ZPP/heme ratios above the normal range, whereas, only 36.4% of the children in the current study (2012) had ZPP/heme ratios higher than the normal range.

While the results presented in Figures 1, 2, 3 and 4 show the changes in PbB and ZPP levels for groups of children living in the Pb-contaminated villages that comprised the study area, Figure 5A,B shows a comparative sample of matched (same) individual children tested

previously for PbB and ZPP/heme levels and re-examined in 2012. This figure illustrates individual declines in current pediatric PbB levels, and a corresponding reduction in ZPP/heme levels for the same child relative to previous test values obtained in 2003, 2006 or 2007. The observed declining trends in PbB levels of matched individual cases shown in Figure 5 are consistent with the reduction in group means over a period of years, as illustrated in Figures 1–4.

Discussion

This study investigated the current Pb exposure and ZPP levels as part of a Pb exposure monitoring program for Andean children living in rural communities with a history of Pb contamination from a local Pb-glazing cottage industry. The results were compared to those of cohorts of children whose Pb exposure levels were assessed over time, beginning in 1996. Previous investigations in the Pb-contaminated study area have shown high levels of Pb poisoning in children living in communities of the study area [17–21]. In addition, pediatric Pb exposure in the study area has been found to be associated with deficits in neurocognitive performance [22–24]. The results of the present investigation showed an overall significant decline in pediatric plumbism in the study area over a period of 16 years. Consistent with the overall group decline in Pb exposure, individual children who were tested in previous years showed reductions in PbB levels. Exceptions to the overall decline in PbB levels were observed in the elevation in mean PbB level for the group of children tested in 1999. It is unlikely that the peak in mean PbB level observed in the 1999 cohort is related to differences in the cohorts tested, because all of children assessed between 1996 and 2012 are from the same study area. The inhabitants of the study area are an essentially a homogeneous, lower socioeconomic group in which most of the families engage in Pb-glazing activities, and are exposed to Pb via similar environmental pathways. Selection bias also does not explain the upswing in PbB levels observed in 1999 since all children examined from 1996 to 2012 were selected without regard to age, gender, educational status, school graded level, or other predetermined selection criteria. While there is no clear evidence to explain the upswing in Pb exposure in this cohort of children, a plausible explanation is that the upswing in PbB levels reflects an increase in community Pb-glazing activities during the period of 1999.

In the past, children in the study area have participated in all aspects of the occupational production of Pb-glazed roof tiles and other ceramics by their families, including Pb extraction, processing, and application of a Pb slurry onto objects, which are then baked in open kilns. The children may be exposed to Pb prenatally from the mother's blood, or postnatally from maternal breast milk during infancy, or later through the ingestion of Pb-contaminated foods and other substances. Further, older children in the study area may be more exposed to Pb through their direct involvement in the production of Pb-glazed ceramics, particularly male children who are typically more active in Pb-glazing activities because of cultural customs. In the 1999 cohort, both males and females showed elevations in mean PbB levels (see Figure 3B). However, for the 1999 cohort, more males (55.2%) than females (30.0%) presented with PbB levels ≥ 45 $\mu\text{g/dL}$, the CDC's recommended level for chelation therapy. This finding suggests that the males, who are more involved with Pb-glazing activities, are driving the peak PbB level for the 1999 cohort as illustrated in Figure 2. In general, the acute Pb exposure level of the children may peak at a particular time, depending on the extent of Pb-glazing activities in the study area.

It is of particular clinical interest to ascertain whether the PbB levels observed reflect recent exposure to Pb or represent chronic Pb exposure. The ZPP level has been used as a biomarker for prolonged or chronic Pb toxicity. In the present study, ZPP/heme and ZPP WB levels were examined, and found to be abnormally elevated (consistent with the PbB

levels), indicating a history of pediatric Pb poisoning and possible Fe deficiency in the study group. Previous investigations in the study area have shown no evidence of pediatric Fe deficiency [28]. However, the Fe status of the children in the study area should be monitored individually. The decline in ZPP levels was found to be parallel to that of the decline in PbB levels, suggesting a reduction in prolonged exposure, and the associated Pb-induced inhibition of heme biosynthesis. PbB and ZPP are measures of Pb in soft tissues, but most absorbed Pb is stored in bone and teeth (in children approximately 70%). In addition to ZPP, bone Pb stores reflect chronic and cumulative Pb exposure. Bone Pb stores may mobilize during pregnancy, breast feeding, bone injuries, or during skeleton growth in children, leading to endogenous Pb release back into blood [10, 12]. While it was not feasible to measure bone-Pb in these field investigations, our previous studies have shown Pb lines in teeth of many children, and elevated levels of Pb in breast milk of nursing mothers in the communities of the study area. Because the serial Pb measurements show essentially a consistent decline in PbB levels over the 16 years from 1996 to 2012, it is unlikely that the results obtained in this investigation are due to bone-Pb stores.

The overall reduction in both PbB and ZPP levels suggests a trend toward declining Pb exposure in the study area, indicating positive outcomes from the earlier introduction of a Pb-exposure education and prevention program, and the therapeutic intervention of succimer chelation therapy [17, 26]. Our Pb-exposure education and prevention program provided information to the families that engage in the production of Pb-glazed ceramics, and to the communities in the study area about the health hazards of occupational Pb exposure [17,18]. Components of the Pb-exposure education and prevention program included improved hygiene techniques that can remove Pb from hands, food, as well as cooking and eating utensils. Our program also emphasized the removal of Pb dust from household objects, removal of Pb-glazed ceramics from the home, and restricting access of children and pregnant women to the highly contaminated soil in the Pb-glazing and baking-kilns areas. Vitamin and mineral supplements were also provided as part of the Pb-exposure education and prevention program. In addition, iron, zinc and multivitamins are provided to the study area as part of the Ecuadorian Ministry of Public Health's Supplementation Program for rural communities. Our previous investigations in the study area have shown that both the Pb-exposure education and prevention program, and succimer chelation therapy have been instrumental in reducing overall Pb poisoning in these communities (26).

The results further suggest that over the years, the population has accepted the principles of our Pb-exposure education and intervention program, and incorporated them into their daily lives. For example, it was reported by the local healthcare providers that many parents are using improved hygienic practices, and have increasingly restricted their children's access to the Pb-glazing work areas. Also, the number of backyard Pb-glazing kilns in the study area has decreased over the past decade, and a new type of lower emission kiln has been introduced to the Pb-glazing communities.

The blood test results were presented to the parents, who were counseled regarding the implications of elevated PbB and ZPP levels in their children. In addition, a community-wide meeting was held in 2012 for families and healthcare providers from the La Victoria community, as well as for officials from the Ecuadorian Ministry of Public Health. Representative families and healthcare providers from the Pb-contaminated communities also attended a 2-day conference at Universidad San Francisco de Quito that examined the health risks of Pb exposure in the study area, and presented the findings of our ongoing studies.

In conclusion, the current study showed a significant decline in PbB levels in the children of the study area relative to previous years. However, the PbB levels of some individual

children remain unacceptably elevated. Because of this finding, it is important to continue to biomonitor the Pb exposure levels of the children living in the Pb-contaminated communities, particularly since Pb-glazing of ceramics remains the primary livelihood of villagers in the study area. In order to further reduce the level of pediatric Pb exposure in this population of Andean children below the U.S. current reference value of 5 µg/dL, for example, there would have to be a complete cessation of Pb-glazing activities, and environmental remediation would need to be initiated in the Pb-contaminated communities.

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Figure 1.

Box plots of blood lead (PbB) level distributions showing declining Pb exposure in Andean Ecuadorian children observed in 5-year intervals (1996–2000, 2003–2007, and 2012). The boxes contain individual PbB levels between the 25th and 75th percentiles. The horizontal lines inside the boxes represent the 50th percentile. The small horizontal lines above the boxes represent the 90th percentile, and the small horizontal lines below the boxes represent the 10th percentile. The individual data points represent cases above the 90th percentile and below the 10th percentile.

Figure 2.

Declining blood lead (PbB) levels over time for individual years from 1996 to 2012 for Andean Ecuadorian children living in Pb-contaminated communities. No PbB tests were performed in the intervening years not listed in the figure. *The asterisks above the year data points in the figure denote that the specified years were significantly different from the reference year, 1996.

Figure 3.

Declining blood lead (PbB) levels over time from 1996 to 2012 as a function of age group (A) and gender (B) for Andean Ecuadorian children living in Pb-contaminated communities. No PbB tests were performed in the intervening years not listed in the figure. *The asterisks above the year data points in the figure denote that for the specified years, the older age group had significantly higher PbB levels than the younger age group.

Figure 4.

Comparison of means (with standard error bars) for (A) blood lead (PbB) levels, and (B) zinc protoporphyrin/heme (ZPP) levels obtained for the years 2006, 2007 and 2012 in Pb-exposed children living in Pb-contaminated communities in the Andes Mountains of Ecuador. Note the parallel decline in PbB and ZPP/heme levels. No PbB or ZPP tests were performed in the intervening years not listed in the figure.

Figure 5.

Comparison of previous blood lead (PbB) levels (A) and zinc protoporphyrin/heme (ZPP) levels (B) obtained in 2003, 2006 or 2007 with current (2012) PbB and ZPP/heme levels in matched individual Andean Ecuadorian children (cases) living in Pb-contaminated communities.

Table 1

Percentage of Ecuadorian Andean children with blood lead (PbB) levels equal to or greater than 5, 10, 45, and 70 µg/dL for the years 1996, 1997, 1998, 1999, 2000, 2003, 2006, 2007 and 2012.

Year	% 5 µg/dL	% 10 µg/dL	% 45 µg/dL	% 70 µg/dL
1996	100	97.2	47.7	20.2
1997	100	93.6	29.0	6.4
1998	100	90.0	25.0	10.0
1999	100	100	42.4	16.9
2000	100	100	20.0	0.0
2003	93.3	76.7	15.6	5.6
2006	98.6	75.7	22.9	5.4
2007	89.7	60.6	9.1	1.2
2012	95.4	68.2	0.0	0.0