Blood Levels of the Heavy Metal, Lead, and Caries in Children Aged 24–72 Months: NHANES III

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Key Words

Blood lead levels · Caries · Heavy metal · NHANES III

Abstract

Lead remains a significant pollutant. It has acute toxic and chronic effects on many tissues and accumulates in teeth and bones. The researchers for this study investigated the association of blood lead levels with the extent/severity of caries as measured by the number of decayed/filled teeth of children aged 24–72 months using data from NHANES III (the Third National Health and Nutrition Examination Survey), accounting for the excess zero caries in the analysis and using less than 2 µg/dl as the reference blood lead level ($n = 3,127$). Zero-inflated negative binomial regression models indicated unadjusted extent/severity mean ratios of 1.79, 1.88 and 1.94 for the number of decayed/filled teeth in children whose blood lead levels were 2–5, 5–10 and >10 µg/dl, respectively, compared with children having <2 µg/dl blood lead levels. The results did not attenuate when other variables were added to the model for the 5–10 and >10 µg/dl levels of exposure. The adjusted extent/severity mean ratios were 1.84, 2.14 and 1.91, respectively, for the categories. This study indicated a strong association of blood lead levels with increasing numbers of carious teeth in children aged 24–72

months. These findings support other studies in an innovative analysis handling cases of children with no caries. The findings may inform caries risk assessment.

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Environmental lead remains a public health concern. Lead exposure has been known to have health consequences for two millennia [Gilbert and Weiss, 2006]. Acute lead poisoning presents with abdominal distress, vomiting, neuropathies, myalgia, weakness, and confusion [Rosin, 2009]. Chronic lead exposure interferes with nerve conduction, enzymes that depend upon calcium, ATPases, oxidative phosphorylation in mitochondria, production of heme, the status of membranes, the metabolism of steroids, and sperm count [Rosin, 2009]. With chronic lead exposure, gingival changes can occur with the development of dark ‘lead lines’ [Rosin, 2009]. There is also concern of a relationship of increased bone lead levels and renal diseases, cataracts, hypertension, and degenerative dementia [Rosin, 2009].

In evaluating blood lead levels for lead exposure, the Centers for Disease Control and Prevention (CDC) used the 97th percentile of blood lead levels in US children in

two consecutive National Health and Nutrition Examination Survey (NHANES) cycles as the upper reference level for children aged 1–5 years [Wheeler and Brown, 2013]. From 2007 to 2010, 2.6% of children (an estimated 535,000) had high blood lead levels (≥ 5 $\mu\text{g}/\text{dl}$) [Gilbert and Weiss, 2006]. Progress in decreasing childhood blood lead levels is occurring. The geometric mean blood lead level was 15 $\mu\text{g}/\text{dl}$ from 1976 to 1980, 3.6 $\mu\text{g}/\text{dl}$ from 1988 to 1991, 1.9 $\mu\text{g}/\text{dl}$ from 1999 to 2002, and 1.3 $\mu\text{g}/\text{dl}$ from 2007 to 2010 [Wheeler and Brown, 2013]. However, the following argument has been made: (1) no blood level of lead is safe, particularly to the developing nervous system of children, (2) blood lead levels of 2 $\mu\text{g}/\text{dl}$ are readily and accurately measurable and, therefore, (3) the upper blood lead level limit should be 2 $\mu\text{g}/\text{dl}$ [Gilbert and Weiss, 2006].

The precipitous drop in lead exposure in children has been attributed to the ban on lead as a constituent of paint (which occurred in 1978), reducing lead in vehicle emissions, remediation of surfaces painted with lead-based paints, remediation of soil in play areas for children, reducing the marketing of products with lead to children, and awareness and monitoring of food such as rice [CDC, 2009; Jaslow, 2013; Wheeler and Brown, 2013]. A source of lead that may affect an unborn child is the maternal blood level of lead (which increases during pregnancy) and lead in breast milk from maternal lead exposure which may have occurred decades earlier [Watson et al., 1997]. Lead has a half-life of roughly 62 years in the body [Watson et al., 1997].

The evidence surrounding the link between lead exposure and dental caries has been reported in previous research motivated by the known accumulation of lead in bones and teeth [Martin et al., 2007; Alomary et al., 2013]. Heavy metals such as lead, cadmium and zinc are assimilated in enamel during mineralization as well as after eruption [Youravong et al., 2005, 2006; Alomary et al., 2013]. Lead has been reported to be able to incorporate into primary teeth from 4 months prenatally until the teeth naturally exfoliate [Gierat-Kucharzewska et al., 2003]. The proposed method is that lead, in its divalent cationic state, substitutes for isovalent calcium sites in dentinal hydroxyapatite [Alomary et al., 2013]. Several studies have attempted to identify tooth tissue concentration differences (enamel vs. dentin; crowns vs. roots) to identify both spatial and temporal deposition rates. Lead exposure and dental caries may have a temporal relationship in that antenatal and early exposure may result in teeth being more vulnerable to caries development. Whereas adults absorb 10% of a dose of lead, chil-

dren absorb 40% and retain 30% while adults retain 1% [Rosin, 2009]. A mechanism by which lead may impact dental caries is the theory that lead interferes with the enzymes for extracellular matrix metabolism and the enamel matrix proteinases involved with amelogenesis; however, one study did not show morphological changes associated with blood lead levels in exfoliated primary teeth [Youravong et al., 2005].

Another means by which lead may influence caries development is through interference with salivary function. In a rat model, there was nearly a 40% increase in caries and a 30% decrease in stimulated saliva from the parotid with lead exposure [Watson et al., 1997]. Without salivary protection, teeth are more vulnerable to dental caries.

Additionally, lead is thought to bind with fluoride in saliva and reduce the bioavailability of the fluoride for remineralization [Moss et al., 1999]. Recently, Youravong et al. [2013] described the relationship of elevated salivary lead levels and a relationship with decreased *Streptococcus mutans* carriage, indicating a possible shift in the oral microbiota that may be related to other cariogenic bacteria flourishing. Salivary lead level detection has been problematic due to methodological issues and salivary collection techniques. Variables in circadian effects on salivary flow, stimulated versus unstimulated whole saliva detection methods, lack of documented reference standards, and low levels of lead within the saliva have adversely impacted current information.

The studies involving lead and dental caries have had equivocal results, with Moss et al. [1999] supporting the relationship, Campbell et al. [2000] not demonstrating that an exposure of >10 $\mu\text{g}/\text{dl}$ as a toddler is a predictor of dental caries in school-aged children, Martin et al. [2007] supporting a weak male-only association of lead exposure with primary tooth caries, and Malara et al. [2006] not finding a significant difference in lead concentration and the roots of carious/noncarious permanent teeth. Study designs had the following significant differences: lead levels were determined using log-transformed lead levels or lead levels dichotomized at 5 or 10 $\mu\text{g}/\text{dl}$; participants had different age categories in the different studies; caries was evaluated on permanent/mixed/primary dentitions, and caries prevalence as decayed and filled primary teeth (dft, presence/absence) was used as an outcome [Moss et al., 1999; Campbell et al., 2000; Martin et al., 2007]. None of the studies adjusted for zero inflation (the high number of children who had no caries) or the extent/severity of caries using modeling of the mean of the number of decayed/filled teeth in the sample. Also, with the geometric

mean of blood lead level at 1.9 µg/dl for 1999–2002 and 1.3 µg/dl for 2007–2010, it may be appropriate to use 2 µg/dl as a reference category in current research [Gilbert and Weiss, 2006].

The aim of this study was to examine the association between caries in children aged 24–72 months and their blood lead levels using a statistical test, zero-inflated negative binomial (ZINB) regression, to determine the relationship. The data to be used are from NHANES III. The hypothesis is that lead is strongly associated with dental caries, and the relationship requires a special analytic technique to properly express that relationship.

Methods

This study involved secondary data analysis of deidentified public access data and did not require institutional review board approval. The NHANES III study, which provided the data, was conducted by the National Center for Health Statistics (NCHS) of the CDC. The NHANES III received institutional review board approval and documented consent was obtained from participants [NCHS, 2012]. NHANES III was a nationally representative survey with a complex survey design. It consisted of interviews of noninstitutionalized US citizens. The interviews included demographic, socioeconomic, dietary, and health-related questions. The survey also had medical and dental examinations, physiological measurement such as height and weight, and laboratory tests on blood and/or urine. The survey was designed to over-sample certain groups (children aged 2 months to 5 years, adults aged 60 years and above, African-Americans, and Hispanics) to provide more reliable statistics [NCHS, 2012]. Further details of the survey are available on the CDC website [NCHS, 2012]. The entire NHANES III sample (1988–1994) included 33,994 participants. The sample had 10,332 children aged from 2 to 18 years [Vargas et al., 1998].

The sample used in this study included children aged 24–72 months who did not have missing data on blood lead levels, dental examination, sex, race/ethnicity, age, poverty index, urban/nonurban status, family's highest education, smoking in the household, birth weight, having been breast-fed, and last dental visit. The sample consisted of 3,127 participants.

The dental examinations for participants were conducted with front-surface mirrors and No. 23 explorers to evaluate all the teeth present [Vargas et al., 1998]. This study used the results of the examinations of dft on tooth-level data of primary teeth. Teeth which were unerupted or missing due to nondisease were coded as such and not considered in the analysis. The National Institute of Dental Research and a dental consultant trained and calibrated the examiners (NHANES, 1988–1994). One of the examiners conducted nearly 50% of the examinations; three others and one back-up examiner conducted the remainder of the evaluations [Vargas, 1998]. A 'gold-standard' examiner observed and replicated evaluations on approximately 30 participants once or twice annually; similarly, each examiner replicated evaluations on a sample of participants within the 6-week period/location (NHANES, 1988–1994). Intra-

and interexaminer calibrations were satisfactory [Vargas, 1998]. The interrater reliability compared reference and examiner; the interrater reliability resulted from repeated examinations and paired observations were used to calculate Cohen's kappa (0.64–1.00 for caries) [Beltrán-Aguilar et al., 2005].

Outcome Variable

The outcome variable was the count of dft – the number of primary teeth with at least 1 decayed or filled surface.

Main Variable of Interest

The main variable of interest was the blood lead level. It was categorized as <2 µg/dl (the reference category), 2–5, 5–10 and >10 µg/dl. The children with blood lead levels below the detection limit of 1 µg/dl (NHANES, 1988–1994) were categorized as <2 µg/dl. NHANES III returned laboratory results below the lower detection limit with a value equal to the detection limit divided by the square root of 2 to distinguish nondetectable laboratory results from detectable results [Vargas et al., 1998].

Other Variables

Other variables in the analysis included predisposing factors such as sex (male vs. female), age (3, 4 and 5 years vs. 2 years as the reference, having the least accumulated exposure) and race/ethnicity (other, non-Hispanic Black and Mexican-American vs. non-Hispanic White as the reference). Enabling factors included in the study were as follows: poverty index (below poverty level vs. at or above poverty level as the reference, having the greater potential to not having been exposed to lead-based paints), urban status (non-urban vs. urban), census region (South, West and Midwest vs. Northeast as the reference, as the West has greater lead deposits, the soil along the lower Mississippi has greater lead content and the Northeast has significant industry), and the highest education level in the family (less than high school and high school graduate vs. more than high school). Lifestyle/behavioral factors included in the study were smoking in the household (yes vs. no as a reference due to the potential of lead exposure from passive smoke), birth weight (<2,500 and >4,100 g vs. 2,500–4,100 g as a reference), breast-fed (yes vs. no as the reference), parent/guardian perception of the condition of the child's teeth (very good, good, fair and poor vs. excellent as a reference), and last dental visit (at least one vs. never as the reference).

Statistical Methods

To account for the NHANES complex survey design, Rao-Scott χ^2 tests were used to examine the associations between blood lead levels and predisposing, enabling and lifestyle/behavioral factors in bivariate analysis.

To account for the high number of children who do not have caries [Böhning et al., 1999], zero-inflated regression methods were applied to account for the excess zeroes. Through graphical comparison of Poisson, negative binomial, zero-inflated Poisson, and ZINB models with the observed data, the ZINB model was determined to provide the best model fit (fig. 1). To explore the influence of the other variables on the relationship between blood lead levels and caries severity, an unadjusted ZINB model was fit, as well as a model adjusting for the other variables listed above. Discussions of the mean severity estimation and standard error calculation for zero-inflated models are presented elsewhere [Preisser et al., 2012].

Fig. 1. Differences in model predictions and observed frequencies for examination of model selection between 4 possible model choices (Poisson, negative binomial, zero-inflated Poisson and ZINB).

SAS version 9.3 (SAS Institute Inc., Cary, N.C., USA) was used for all descriptive statistics and mean severity ratio calculations. Additionally, STATA 12.1 (StataCorp LP, College Station, Tex., USA) was used to apply the ZINB models, with special consideration given to the complex multistage sampling scheme of NHANES III. Software code and necessary ZINB model information for the mean severity calculations are provided in the online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000365297).

Results

There were 3,127 participants. The survey-weighted mean number of teeth with caries/restorations was 1.03 (standard error 0.09). In this sample, approximately 76% of the children had no caries, 13% had 1–3 dft and 11% had >3 dft.

Table 1 has data on the sample characteristics. The sample was 51.90% male and 62.54% non-Hispanic White. The families were generally above the poverty line (70.56%), had at least 1 member who was at or above

a high school education (74.84%) and did not have smoking in the household (62.03%); 8.5% of the children had a birth weight <2,500 g. The children were evenly distributed in age (2–5 years), urban/nonurban status, having been breast-fed, and having had a dental visit. The blood lead levels were <2 µg/dl for 28.19% of the participants and between 2 and 5 µg/dl for 48.29% of the participants.

Table 2 has the predisposing, enabling and lifestyle/behavioral variables by the exposure of interest (blood lead level categories). There were significant associations between higher blood lead levels and age, race/ethnicity, census region, living below the poverty level, living in a household where the highest education was less than a high school education, living in a household where smoking occurred, low birth weight, reported condition of teeth, and dental visit. The associated weighted percentages of >10 µg/dl were 45.10% (for non-Hispanic Blacks), 62.69% (for living under the poverty level), 51.79% (for living in a household where the highest education was less than a high school education), 74.96% (for bottle-feed-

ing), and 54.71% (for living in a household where someone smoked).

Table 3 has the overall mean severity ratios from both the unadjusted and adjusted ZINB models. For the blood lead group of 5–10 µg/dl, the overall adjusted mean severity ratio was 2.138, indicating that children in this blood lead group had 113.8% more caries on average than children with a blood lead level of <2 µg/dl. The adjusted mean severity ratios were 1.840, 2.138 and 1.906, respectively, for the categories of 2–5, 5–10 and >10 µg/dl for dft compared with children with blood lead levels <2 µg/dl, adjusting for sex, race/ethnicity, age, urban status, census region, poverty level, highest household education, smoking in the household, birth weight, whether the child was breast-fed, and whether the child visited the dentist.

Discussion

The results of this study indicate a strong independent association of lead with the severity/extent of caries in children aged 24–72 months using a statistical technique to account for the inflation of values due to the high number of children with no caries. The unadjusted severity/extent mean ratio for children with >10 µg/dl was 1.937, and the adjusted value was 1.906. Among the blood lead groups of 2–5 and 5–10 µg/dl, the relationship was strengthened by adjusting for the other variables. This strengthening of association was not observed in the highest exposure group, although the effect remained significant. The survey-weighted mean number of teeth with caries/restorations was 1.03 (standard deviation 0.09), with approximately 76% of children having no caries.

This is the first study to examine the severity/extent mean ratio between lead and caries using transformations of zero-inflated modeling techniques to obtain overall effect estimates of blood lead exposure on caries severity. One of its strengths is that it was from a large, national survey in which calibrated investigators used validated and reliable questionnaires. Oral evaluations for dft and laboratory procedures for blood lead levels were also calibrated. A limitation of the study, and any study using a cross-sectional study design, is that temporality is a concern. This study supports a previous study of NHANES III data which examined the prevalence of caries in children aged 2 years through to 12 years and found an association of decayed, missing and filled surfaces in primary and permanent teeth [Moss et al., 1999]. Other studies of

Table 1. Description of NHANES III subsample characteristics (n = 3,127)

	Number	Weighted %
Blood lead levels, µg/dl		
<2	594	28.19
2–5	1,500	48.29
5–10	785	18.44
>10	248	5.08
Sex		
Female	1,561	48.10
Male	1,566	51.90
Race/ethnicity		
Non-Hispanic White	897	62.54
Non-Hispanic Black	1,017	16.47
Mexican-American	1,048	9.76
Other	165	11.23
Age, years		
2	795	23.52
3	762	24.86
4	801	24.39
5	769	27.23
Poverty index		
Below poverty	1,338	29.44
At or above poverty	1,789	70.56
Urban		
Urban	1,611	47.83
Nonurban	1,516	52.17
Census region		
Northeast	322	15.97
Midwest	548	23.16
South	1,400	35.79
West	857	25.08
Highest education in household		
Less than high school	1,160	25.16
High school graduate	1,032	33.35
More than high school	935	41.49
Smoking in household		
Yes	1,164	37.97
No	1,963	62.03
Birth weight, g		
<2,500	283	8.53
2,500–4,100	2,621	83.08
>4,100	223	8.39
Breast-fed		
Yes	1,383	53.85
No	1,744	46.15
Parent/guardian perception of the condition of the child's teeth		
Excellent	1,041	40.12
Very good	690	23.37
Good	913	25.49
Fair	313	7.12
Poor	170	3.90
Last visit to dentist		
Never	1,837	53.06
At least once	1,290	46.94

Table 2. Blood lead levels and variables of interest: NHANES III

	<2 µg/dl		2–5 µg/dl		5–10 µg/dl		>10 µg/dl		p value
	number	weighted %	number	weighted %	number	weighted %	number	weighted %	
Sex									0.4760
Female	315	49.03	759	48.71	385	46.88	102	41.63	
Male	279	50.97	741	51.29	400	53.12	146	58.37	
Race/ethnicity									<0.0001
Non-Hispanic White	289	73.89	453	64.87	130	47.87	25	30.75	
Non-Hispanic Black	101	6.56	434	15.10	334	27.29	148	45.10	
Mexican-American	168	7.59	544	10.43	274	11.37	62	9.55	
Other	36	11.95	69	9.60	47	13.47	13	14.60	
Age, years									<0.0001
2	117	18.85	348	22.10	252	32.71	78	29.64	
3	134	21.94	393	27.57	177	23.03	58	21.97	
4	155	25.42	388	23.86	195	23.54	63	26.75	
5	188	33.79	371	26.47	161	20.71	49	21.64	
Poverty index									<0.0001
Below	110	11.64	590	28.88	460	48.96	178	62.69	
At or above	484	88.36	910	71.12	325	51.04	70	37.31	
Urban status									0.8840
Urban	298	48.94	728	46.17	426	49.57	159	51.14	
Nonurban	296	51.06	772	53.83	359	50.43	89	48.86	
Census region									<0.0001
Northeast	42	10.34	120	13.25	116	27.11	44	32.72	
Midwest	94	21.38	195	20.83	152	27.66	107	38.78	
South	256	33.60	730	39.40	352	33.76	62	21.04	
West	202	34.68	455	26.52	165	11.47	35	7.47	
Highest education in household									<0.0001
Less than high school	112	12.90	537	24.57	378	38.10	133	51.79	
High school graduate	200	31.91	489	33.55	266	36.81	77	26.82	
More than high school	282	55.20	474	41.87	141	25.09	38	21.39	
Smoking in household									<0.0001
Yes	145	24.57	548	40.70	334	46.69	137	54.71	
No	449	75.43	952	59.30	451	53.31	111	45.29	
Birth weight, g									0.0155
<2,500	37	6.42	124	7.90	90	11.64	32	14.93	
2,500–4,100	507	83.29	1,261	83.72	654	82.24	199	78.84	
>4,100	50	10.28	115	8.38	41	6.13	17	6.23	
Breast-fed									<0.0001
Yes	333	62.48	699	56.20	289	42.47	62	25.04	
No	261	37.52	801	43.80	496	57.53	186	74.96	
Parent/guardian perception of the condition of the child's teeth									0.0025
Excellent	249	45.85	497	39.96	218	33.82	77	32.82	
Very good	144	25.86	322	23.08	173	21.17	51	20.32	
Good	136	19.63	449	25.80	257	32.24	71	30.56	
Fair	47	6.84	141	6.39	90	8.79	35	9.47	
Poor	18	1.82	91	4.77	47	3.98	14	6.84	
Last visit to dentist									<0.0001
Never	291	45.98	861	52.98	524	61.41	161	62.86	
At least once	303	54.02	639	47.02	261	38.59	87	37.14	

Weighted percentages were obtained to control for complex sample design, therefore the division of individual cell sizes by the total sample will not reflect weighted percentages. Significant group differences were tested by χ^2 statistics.

Table 3. Overall mean severity ratio estimates for dft count in children aged 24–72 months: NHANES III (n = 3,127)

Variables	Unadjusted	Adjusted ¹
Blood lead levels, µg/dl		
<2	1.000	1.000
2–5	1.788 (1.191–2.685)	1.840 (1.355–2.499)
5–10	1.878 (1.249–2.822)	2.138 (1.363–3.355)
>10	1.937 (1.186–3.165)	1.906 (1.170–3.106)
Sex		
Female		1.000
Male		0.848 (0.678–1.059)
Race/ethnicity		
White		1.000
Black		1.451 (0.977–2.156)
Mexican-American		1.975 (1.382–2.824)
Other		2.528 (1.349–4.739)
Age, years		
2		1.000
3		3.441 (1.921–6.162)
4		4.875 (2.794–8.506)
5		8.633 (5.294–14.078)
Urban status		
Urban		1.000
Nonurban		1.384 (1.043–1.836)
Census region		
Northeast		1.000
South		1.045 (0.605–1.805)
West		1.337 (0.785–2.276)
Midwest		1.216 (0.698–2.120)
Poverty index		
At or above poverty level		1.000
Below poverty level		1.119 (0.788–1.589)
Highest family education level		
Less than high school		0.957 (0.581–1.576)
High school		1.000
More than high school		0.920 (0.587–1.443)
Smoking in household		
No		1.000
Yes		1.818 (1.406–2.349)
Birth weight, g		
<2,500		0.752 (0.394–1.435)
2,500–4,100		1.000
4,100		0.793 (0.441–1.425)
Breast-fed		
No		1.000
Yes		1.130 (0.851–1.500)
Dental visit		
No		1.000
Yes		1.559 (1.104–2.201)
Parental perception of oral health		
Excellent		1.000
Very good/good		4.182 (2.650–6.599)
Fair/poor		25.864 (16.863–39.668)

Values in parentheses are 95% confidence intervals.

¹ Mean severity ratios are estimated from ZINB regression with all co-variables in each part of the model, using survey-weighted mean levels of all other variables.

caries and lead exposure involved older children. One study indicated that urban children aged 6–10 years with increased blood lead levels had an association with caries; another study indicated a relationship of blood lead level and caries only with males, and only in primary teeth, and another indicated no increased association of decayed, filled and missing teeth with blood lead levels early in life (18–37 months) [Bellinger, 2011]. This study adds to the literature a strong association between lead exposure and severity/extent of caries in children aged 24–72 months. Even observed differences between <2 and 2–5 µg/dl indicate support for lower limits of risk with lower exposure.

Future research is needed into the development of a quick salivary lead detection test [Youravong et al., 2013] which could be used by dentists in the routine care of children to determine high lead exposures, refer for follow-up care and use to inform caries risk assessment. Caries progression is a concern and dentists need to be aware of factors beyond the traditional risk factors for early childhood caries and to consider those risks in caries risk assessments. Future research is also needed to investigate lead exposure to specific populations. Children immigrating to the USA from China, where rice contamination with lead is high, may be at increased risk of lead poisoning and associated caries. Children emigrating from emerging market countries may be at greater risk of exposure to lead from industrial emissions, fossil fuel burning, waste incineration, consumer products, mining, and smelting due to limited regulatory oversight [Horton et al., 2013]. There is a high caries incidence in Native American children. Do these children have a higher lead exposure? The current research sample was not powered to evaluate subpopulations. Most of the lead exposure in the USA is from pockets of low-income, urban neighborhoods with older housing [Horton et al., 2013]. Policy research is also needed to determine a means of lead abatement in housing in which lead paint was not removed or contained after the 1978 federal ban on lead paint [Korfmacher and Hanley, 2013]. The researchers for the CDC state that primary prevention, the assurance that all homes are lead-safe, is the only practical approach to preventing elevated blood lead levels, and prevention policies should be targeted at the children most at risk [Wheeler and Brown, 2013].

Despite considerable efforts, the problem of lead has not disappeared, and the threshold level of safety has not been determined [Bellinger, 2011]. This study indicates that even low blood lead levels are associated with increased dental caries extent/severity in early life.

Acknowledgment

The research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health (Award No. U54GM104942). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. R.C.W.

and D.L.L. conceived and designed the study; R.C.W. and R.J.J. conducted the literature search; D.L.L. analyzed the data, and R.C.W., R.J.J. and D.L.L. wrote the paper.

Disclosure Statement

The authors have no conflicts of interests to declare.

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