

TITLE: Perinatal Lead Exposure and Risk of ASD: a County-level Natural Experiment in 10 US States

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Abstract

Prevalence of autism spectrum disorder (ASD) is continuing to rise unabated worldwide; however, the origins of this epidemic and the drivers of its sustained momentum remain elusive. While exposure to air pollution and abnormal metal levels in children with ASD have been identified as environmental risk factors of ASD, these studies have failed to explain the emergence of the epidemic. Paramount to protecting against future cases of this neurodevelopmental disorder is a better understanding of its etiology. Here, we demonstrate in a series of natural experiments at the US state county level, that perinatal lead exposure is a potent inhibitor of future diagnosis of autism. These results contrast with how lead is typically perceived, namely, as detrimental to normal neurodevelopment. Lead's disuse in the last several decades, while benefiting the physiological health of children in many ways, has likely prompted the current epidemic by leaving infants more exposed to other metal toxicants.

1 Background

Autism Spectrum Disorder (ASD) is a neurodevelopment disorder manifested in infancy.

Though prevalence of ASD is ever-increasing it remains a matter of dispute as to whether there is an epidemic or if the increasing prevalence is merely coming to epidemiological terms with a condition that has always been endemic (1). Disparities in prevalence from studies conducted with different study years, ages groups, or geographies, are often rationalized to result from cultural differences or study methodologies (1,2). However, some researchers have sought an environmentally-based rationale to understand the epistemology of the epidemic (3).

The prevalence of autism has been rising since the earliest estimates presented in the 1970s (3.1 per 10,000) (4). Though the methods in each study differ, in recent years ASD prevalence has exceeded 2% in more developed nations such as Australia, South Korea, Sweden, and USA and more than 3% in Japan (5–9). Meanwhile other countries—recently developed or developing—such as Bangladesh, Croatia, Ecuador, Oman, Taiwan, and Venezuela, have seen in the last couple of decades rates well below 1% (10–15). A striking example of the disparity is the difference in prevalence in children from Oman (1.4 per 10,000, aged 0-14 years) and Goyang City, South Korea (264 per 10,000, aged 7-12 years) within rather contemporaneous studies (6,16).

Though the etiology of ASD may be uncertain, we can surmise that a satisfactory explanation for the autism epidemic would account for three main discrepancies: 1) temporal differences in prevalence among successive study years using similar methodologies; 2) higher prevalence among younger cohorts within single-year screening studies; and 3) geographical differences in prevalence which can vary widely not just on the national level, but also on more regional scales

(17). Though some studies have identified environmental risk factors for ASD, none have satisfactorily met these criteria to explain the onset and persistence of the epidemic.

Studies investigating exposure to toxic metals have been marked by inconsistency (18–21).

Notably, investigations into perinatal metal exposures are lacking, as this requires tracking a large cohort over several years. However, in a case-control study by Schmidt et al. (2014), maternal perinatal iron (Fe) supplementation, as retrogradely reported, was found to be associated with reduced risk of ASD in offspring (22). We have previously suggested that if one considers the context of a decreasing prevalence of Fe deficiency in infants, women of child-bearing age, and pregnant women, it is doubtful that this finding can be explained by maternal Fe deficiency. Crucially, Fe may have been acting as an effect modifier, decreasing exposure to another potentially toxic metal (23). The molecular mechanism behind this regulation of metal transportation is well studied. Specifically, the divalent metal transporter-1 (DMT1) is implicated in mediating gut absorption of metals. Low blood Fe levels can lead to upregulation of intestinal DMT1 which enables increased absorption of other metals (24). Conversely, if levels of Fe are increased, downregulation of DMT1 places other metals at a disadvantage (25). The significance of DMT1 is further enhanced by its presence in the placenta and mammary gland which may impact the developing fetus and infant, respectively (26).

The above hypothesis suggests the possibility that metals other than Fe may similarly downregulate DMT1 or other metal transporter. Counterintuitively, a steep decline in exposure to lead (Pb) over the past few decades, due to concern over neurodevelopmental impairment, may have inadvertently led to the dramatic rise of ASD. Inoculation to ASD from maternal exposure to Pb may have prevented higher exposure to the unidentified metal within the developing fetus and infant, thus preventing the onset of ASD. With the bulk source of Pb pollution coming from

leaded gasoline, legislation to eliminate this pollutant has led to dramatically lower blood lead levels (BLL). The effectiveness of these measures can be seen in the decline in mean childhood BLL. For instance, mean childhood BLL dropped in the US from 16 $\mu\text{g/dL}$ in 1976 to 3.2 $\mu\text{g/dL}$ in 1994 (27). By 2018, median BLL was 0.6 $\mu\text{g/dL}$ in children aged 1-5 years (28).

A satisfactory explanation for the ASD epidemic would resolve the temporal and geographic discrepancies in ASD prevalence. This could be manifested not just at the national level, but also within geographic borders where generally homogenous methods for diagnosing ASD exist. With this understanding, the following studies attempt to explain the variation in ASD prevalence at different scales and across time by considering differences in Pb exposures.

2 Methods

To examine the nature of perinatal Pb and other exposures in relation to observed prevalence of ASD we conducted ecological studies in 10 individual states at the county or municipality level. The benefit of using an ecological model to measure the effect of Pb exposure on ASD is the wide variation observed across large, aggregated areas that may not be available at the individual level within limited geographical areas. Also beneficial is the ability to observe historical levels of Pb which are no longer present in developed countries.

States were selected based on total population and data availability. This was followed by an aggregated study involving a selection of these states. Due to the wide variability in these metrics, data extraction methods differed with each state (details of each study are below).

However, four items were sourced from each state at the county or municipality level: (1) ASD prevalence, (2) a proxy of perinatal BLL, (3) population density, and (4) GDP per capita.

The Individuals with Disabilities Education Act (IDEA) requires that states track the number of students with autism requiring disability services due to any perceived educational impact. It is therefore possible that a student may have clinically diagnosed ASD but not qualify for autism under IDEA. Thus, estimation using only school-based records may be lower than estimates using both health and educational records (29). Except for New Jersey, annual prevalence of ASD was determined for children aged six years or in first grade, based on state-provided student ASD counts which were divided by total student enrollment in respective years. This age group was chosen based on an increased likelihood of specificity of ASD compared to other age groups, and is often accompanied by greater symptom severity (30).

As a child's BLL correlates well to that of their mother and due to the high number of children tested annually within each state, elevated BLL ≥ 5 $\mu\text{g/dL}$ (EBLL-5) among children served as a surrogate of perinatal BLL (2,3). These data were sourced annually by the state for select years which corresponded to the birth years of students used to derive ASD prevalence (i.e., six years prior to student enrollment year). While children's BLL is typically lower than adult BLL, it was necessary to determine only the relative BLL among states.

As it was essential to understand the putative inhibitory effects of Pb within the context of exposure to ASD-causing toxicants, and with vehicle emissions potentially being the main source of this pollutant, we used population density as a proxy indicator (31). Pollutants from vehicle emissions may include not just tailpipe emissions, but also emissions from tires, brakes, and road surface (32). Mean ZIP-code tabulation area (ZCTA)-level population density (persons per square mile [PPSM]) was weighted by population. These data were derived for each state from US census year 2010, which was extracted from the Missouri Census Data Center (33).

With the exception of New Jersey, GDP per capita income levels, which served as a socioeconomic status indicator, were sourced from the U.S. Census Bureau, from American Community Survey 2009-2013 5-Year Estimates at the county or municipality level (34).

Linear regression models for each state were fitted with EBLI only, adjusted for population density, and then adjusted for year and income. In separate models, population density was treated as a monotonic variable and as a quadratic expression.

2.1 Single state county/municipality-level analyses

California.

Annual school enrollment of students with ASD aged 6 years was sourced from the Californian Department of Education for school years 2013-2014 through 2018-2019, for each county with a count of ten or more students (Table 1) (35). To derive ASD prevalence, ASD student count was divided by total student enrollment for corresponding age or, if not available (school year 2013-2014), enrollment within first grade. Counties with a minimum of 600 students by age and school year were used in analyses. Annual percentage of children aged 6-21 years with an EBLI of ≥ 4.5 $\mu\text{g/dl}$ (hereafter, EBLI-5) was sourced from the Californian Department of Public Health for years 2007-2012 in all counties (36).

Illinois.

Annual school enrollment of students with ASD in first grade was sourced for each county from the Illinois State Board of Education for school years 2010-2011 through 2020-2021 (37). To derive ASD prevalence, ASD student count was divided by total first grade student enrollment for the corresponding school year. Counties with a minimum of 600 students by age and school year were used in analyses. Annual percentage of children aged ≤ 6 years with an EBLI of ≥ 5 and ≥ 10

µg/dl was sourced from the Illinois Department of Public Health for years 2008-2014 and 2004-2014, respectively (38).

Massachusetts.

Annual school district-level total enrollment and students with ASD in first grade was sourced from the Massachusetts Department of Elementary and Secondary Education for the 2009-2010 through 2020-2021 school years (39). All school districts located within the same municipality were combined; vocational districts were excluded from analysis owing to their high degree of student insourcing from municipalities located outside the home district. Municipalities with a minimum of 600 students by age and school year were used in analyses. The annual percentage of children aged <6 years with EBL ≥ 5 ug/dl in 2003-2014 at the municipality level was sourced from the Massachusetts Executive Office of Health and Human Services (40). The following algorithm was used to determine EBL for each municipality: 1) EBL was derived by first obtaining the combined (i.e., confirmed and unconfirmed) 5-10 ug/dl EBL rates for all children; 2) If any of these data were censored, just the unconfirmed rates or confirmed rates (in that order) for all children were then applied; 3) If all of the above were unavailable, then female only or male only rates were applied towards the final EBL rate in the same pattern of availability (i.e., combined was first followed by unconfirmed and then confirmed totals); 4) Thereafter, the rate of children with EBLs >10 ug/dl was determined and added to the 5-10 ug/dl EBL rate. For school districts that encompassed multiple municipalities, rates of elevated EBL were weighted by municipality population.

Minnesota.

Annual school enrollment of students with ASD aged 6 years was sourced for each county from the Minnesota Department of Education for school years 2011-2012 through 2018-2019 (41). To

derive ASD prevalence, ASD student count was divided by total student enrollment in the first grade for the corresponding school year. Counties with a minimum of 500 students by age and school year were used in analyses. The annual percentage of children aged ≤ 6 years with a EBLL of ≥ 5 $\mu\text{g/dl}$ was sourced from the Minnesota Department of Health (MDH) and the Minnesota Legislative Reference Library for years 2005-2012 for all counties (42,43).

Missouri.

Annual school enrollment of students with ASD aged 6 years was sourced for each county from the Missouri Department of Elementary and Secondary Education for school years 2006-2007 through 2018-2019 (44). Student counts below 10 were suppressed. To derive ASD prevalence, ASD student count was divided by total student enrollment for the first grade in the corresponding school years. Counties with a minimum of 600 students by age and school year were used in analyses. Annual percentage of children aged < 6 years with a EBLL of ≥ 5 $\mu\text{g/dl}$ was sourced from the Missouri Department of Health and Senior Services for years 2000-2012 for all counties (45). Both confirmed and unconfirmed blood lead levels were incorporated to derive rates.

New Jersey.

Total enrollment of students with autism was obtained from the New Jersey Department of Education for the 2007-2008 school year at the municipality (i.e., by school district) level for children aged 6-8 years (46). This was divided by total student enrollment for grades 1-3 in the same school year to derive the ASD prevalence for each municipality. All three years were collected into one total to increase the population totals for each municipality, thus improving the accuracy of the derived prevalence. Municipalities with a minimum of 600 students in total were used in analyses. EBLL > 10 $\mu\text{g/dl}$ in infants 6-29 months at the municipality level was sourced from the New Jersey Department of Health and Senior Services annual report on childhood lead

poisoning for the fiscal year 2002 only (July 1, 2001 – June 30, 2002) (47). Although this year corresponded to a period 0-3 years prior to birth for students aged 6-8, EBL-10 was unavailable for years prior to 2002. Income per-capita was at the county level and sourced from the New Jersey State Data Center 2000 Census (48).

Ohio.

Annual total county-level enrollment of students with ASD in first grade for school years 2015-2016 through 2019-2020 was sourced from the Department of Education; only counties with a minimum of 10 students with ASD were available for extraction (49). This was divided by corresponding annual total first grade student enrollment to derive the prevalence of students with ASD. Counties with a minimum of 500 students by age and school year were used in analyses. Annual county-level confirmed and unconfirmed EBL >5 ug/dl for children aged <6 years for years 2009-2013 was sourced from the Ohio Department of Health (50).

Pennsylvania.

Annual total enrollment at the county level of students with ASD aged 6 years in 2010-2011 through 2019-2020 school years was sourced from the Pennsylvania Department of Education for all counties in which there were >10 students with ASD (51). This was divided by the total student enrollment of children in first grade in corresponding school years to derive the prevalence of students with ASD. Counties with a minimum of 600 students by age and school year were used in analyses. Annual EBL ≥ 10 ug/dl for children aged ≤ 6 years was sourced for the years 2004-2013 from the Department of Public Health at the county level (52).

Texas.

Annual total enrollment at the county level of students with ASD aged 6 years in 2019-2020 through 2020-2021 school years was sourced from the Texas Education Agency for all counties in which there were >10 students with ASD (53). This was divided by the total student enrollment of children aged 6 years in corresponding school years to derive the prevalence of students with ASD. Counties with a minimum of 600 students by age and school year were used in analyses. Annual EBLI ≥ 5 ug/dl for children aged ≤ 5 years was sourced for the years 2013-2014 from the Texas Department of State Health Services at the county level(54).

Wisconsin.

Annual total county-level enrollment of students with ASD in first grade in each school year from 2005-2006 through 2019-2020 was sourced from the Wisconsin Department of Public Instruction for all counties in which there were >10 students with ASD (55). This was divided by the number of students in first grade in corresponding school years to derive the prevalence of students with ASD. Counties with ≥ 600 students in 1st grade in each school year were used in analyses. Annual EBLI ≥ 5 ug/dl for children aged ≤ 16 years was sourced for years 1998-2014 from the Wisconsin Department of Health Services at the county level (56).

2.1.1 Aggregated county/municipality-level analyses

Multistate linear regression models were developed consisting of state counties/municipalities (from individual state analyses above) with EBLI-5 data; therefore, New Jersey and Pennsylvania were excluded. As each state had its own timeframe of analysis, years of study were only partially overlapping. Only counties/municipalities with observations of ≥ 600 students were incorporated into the models. Annual prevalence of ASD was plotted against EBLI-5 and population density.

Models were fitted with EBLL-5 only and then adjusted for population density, year, and income. Both linear and quadratic models were fitted to consider a nonlinear relationship between population density and ASD. Additional models were fitted with various thresholds of population density (≥ 2000 , ≥ 4000 , and ≥ 6000 PPSM).

Due to the potential impact in the transition from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) to the fifth edition (DSM-5), subsequent regression models were fitted which employed the years prior to and after publication of DSM-5 (i.e., 2005-2014 and 2014-2020, respectively).

2.2 Statistical Analysis

Adjusted coefficient of determination (R^2) was used to provide a measure of the explanatory power of the models. All regressions analyses were ordinary least squares and were performed with SAS University Edition. Models were fitted through a backward and forward stepwise selection process. Significance was set at $p < 0.05$, however, in relevant models, non-linearity was considered significant at $p < 0.01$. To evaluate the existence of geographical patterns in Pb exposure or the prevalence of ASD, we represented each year of study in choropleths of the US. Annual prevalence of ASD was plotted by EBLL and population density.

3 Results

3.1 Single state analyses

Mean ASD prevalence, EBLL incidence, and population density across each individual state study period are provided in Table 2. Annual choropleths of EBLL-5 incidence and ASD prevalence for each state are provided in Supplement 1. The number of observations (i.e., years

in which ASD prevalence and corresponding EBLL are present and in which the total student count meets the requirements) for each state ranged from 59 in New Jersey to 375 in Wisconsin.

The largest range in mean ASD between first and final years was in Massachusetts, with 1.33% having ASD in 2009 and 3.21% in 2020. Massachusetts also had the largest intra-year range of ASD prevalence in both the first (0.69-2.85%) and final (1.67-6.19%) years of study. Missouri had the largest drop in EBLL-5 from the first (36.7%) to last (4.0%) years of the study. Mean population density ranged from 897 PPSM in Minnesota to 8407 PPSM in Massachusetts.

Table 3 presents the correlations of EBLL (i.e., EBLL-5 or EBLL-10) with population density and income. In six of the ten states, EBLL was positively correlated with population density. In contrast, EBLL was negatively correlated with income in seven states.

Regression models for each state are displayed in Table 4. On univariate analysis, each state, except for Ohio ($P = 0.2205$), displayed a significant inverse association of EBLL (i.e., EBLL-5 or EBLL-10) with ASD prevalence. Univariate adjusted R^2 was highest in Massachusetts, with EBLL-5 alone explaining 37% ($n = 206$, $R^2 = 0.3701$, $P < 0.0001$) of model variance.

A monotonically treated population density covariate was then fitted to each model, in addition to EBLL. Population density was positively associated with ASD in California ($P = 0.0094$), Illinois ($P = 0.0002$), Minnesota ($P = 0.0004$), Ohio ($P = 0.0301$), Pennsylvania ($P < 0.0001$), and Wisconsin ($P < 0.0001$). In contrast, population density was negatively associated with ASD in Missouri ($P = 0.0029$) and was trending towards a negative association in Massachusetts ($P = 0.0689$). Upon controlling for population density, year, and income, EBLL remained associated with decreased risk of ASD in California ($P = 0.0022$), Massachusetts ($P = 0.0365$), New Jersey ($P = 0.0185$), and Texas ($P = 0.007$). Income was positively associated with ASD in

Massachusetts ($P = 0.0449$), Missouri ($P < 0.0001$), and New Jersey ($P = 0.01$), while negatively associated with ASD in Ohio ($P = 0.0055$).

A quadratically treated population density covariate was then fitted to each model (Table 5).

Population density was nonlinearly associated with ASD in Illinois ($P = 0.0021$), Minnesota ($P = 0.0276$), and Pennsylvania ($P = 0.0015$), with each state having an inversely U-shaped association. In contrast to the preceding states, Ohio ($P < 0.0001$) and Texas displayed U-shaped quadratic associations ($P = 0.0172$). After controlling for year and income, Wisconsin ($P = 0.0202$) was also shown to have significant quadratic associations of population density with ASD. With a quadratically shaped population density, ASD was also negatively associated with income in California ($P = 0.0458$).

3.2 Aggregated analyses

Several models were developed with aggregated county/municipalities from a selection of states, each with EBLT thresholds of ≥ 4.5 or $5.0 \mu\text{g/dl}$. As Illinois had EBLT threshold of both ≥ 5.0 and $\geq 10.0 \mu\text{g/dl}$, the state was included in the aggregated models. New Jersey and Pennsylvania were excluded from all models owing to lack of EBLT-5 availability. Criteria and characterization of each model are displayed in Table 6.

Eight states were included in the first aggregated analysis (Model #1, $n = 1564$). Annual choropleths of ASD prevalence and EBLT-5 incidence are presented in Figures 1 and 2 for all states excluding Massachusetts. Annual characteristics are presented in Table 7 and scatterplots of all observations are depicted in Figure 3. Fitted with EBLT-5 and population density as the only independent covariates, and applying a quadratic assumption for population density, 27% of variance was explained ($R^2 = 0.2691$, $P < 0.0001$, Table 8). With the exclusion of Massachusetts (i.e., Model #2), 23% of the variance of the model was accounted for ($R^2 = 0.2288$, $P < 0.0001$).

In each model, the inverse relationship observed with EBL-5 was maintained after controlling for year and income ($P < 0.0001$, for each model). Furthermore, income was not associated with ASD in either model ($P = 0.5369$ and $P = 0.4246$, respectively).

Models were also fitted at increasing thresholds of population density: ≥ 2000 PPSM (adj. $R^2 = 0.3691$, $P < 0.0001$, $n = 466$), ≥ 4000 PPSM (adj. $R^2 = 0.4943$, $P < 0.0001$, $n = 257$), and ≥ 6000 PPSM (adj. $R^2 = 0.5808$, $P < 0.0001$, $n = 178$). EBL-5 was significantly associated with decreased risk of ASD in each model. Again, controlling for year and income did not alter the significant inverse relationship between EBL-5 and ASD. While population density was positively associated with increased risk of ASD in the least restrictive models (i.e., ≥ 0 and ≥ 2000 PPSM), as population density threshold increased in the most restrictive models (i.e., ≥ 4000 and ≥ 6000 PPSM), its association with ASD failed to reach significance. We then combined the most restrictive population density (i.e., ≥ 6000 PPSM) with increased total student count thresholds of ≥ 1000 ($n = 123$) and ≥ 2000 ($n = 71$); this had the effect of further increasing the explanatory power of the models (adj. $R^2 = 0.6189$ and 0.7391 , respectively). Notably, unlike prior models, controlling for school year in the latter three models failed to produce a significant relationship between year and ASD.

As the DSM transitioned from the fourth to fifth editions, with accompanying change in criteria of ASD, pre- and post-publication years were modeled separately. Results of the 2005-2013 and 2014-2020 models were similar. BLL maintained a negative association for each model and population density displayed a nonlinear association with ASD. Interestingly, income was positively associated with ASD in the former model but was negatively associated with ASD in the latter model.

4 Discussion

This is the first series of population-level studies investigating the effect of perinatal exposure to Pb on the subsequent risk of ASD. These natural experiments involved geographically disparate regions across the US, at different scales, and over varying time periods. Pb was found to be associated with diminishment of ASD prevalence at both the county and municipality levels. The weight of evidence is highly suggestive that the relationship between Pb and ASD is not merely associative, but also causal in nature, and demonstrating that Pb is a potent inhibitor of ASD. Overall, it indicates that the deliberate pursuit in the decline of Pb in the built environment over recent decades due to concerns over toxicity has paradoxically, and tragically, generated the current autism epidemic.

Few studies measuring maternal Pb levels and subsequent risk of ASD have been conducted. Results from the current study align with one recent prospective Norwegian case-control study in which maternal blood, sampled at 17 weeks of gestation, was retrospectively investigated for various metals (57). Though confidence intervals overlapped, uncontrolled mean Pb from mothers with ASD children ($n = 397$; $8.35 \mu\text{g/L}$, 95% confidence interval [CI]: 7.97, 8.75) was trending lower than those in the control group ($n = 1034$; $8.82 \mu\text{g/L}$, 95% CI: 8.60, 9.05) (57). The relatively small gap between case and control Pb levels may only be indicative of Pb not being the only determinant of ASD. For instance, as we have seen in the current study, population density is also strongly deterministic.

In contrast to the above, in a perinatal study of dental metal levels, Arora et al. found increased Pb among ASD twins compared to non-ASD siblings (58). However, altered dental enamel—marked by the presence of accentuated lines caused by physiological stress or

undernourishment—has also been found in children with autism (59). Such early dysregulation continuing through childhood may confound dental analyses. Metal studies using other matrices may be similarly confounded by altered bone development—a critical depository of Pb and other metals—in children with ASD (60). This may explain why postnatal studies of Pb have had mixed results, with some findings being in contrast to the present study(61–63), while others are more aligned (64,65).

BLL peaked in the 1970's at a mean level above 15 ug/dl, however, Pb exposure from the built environment has been a constant for decades preceding this (66). A major source of Pb exposure was introduced after its discovery as a gasoline antiknock agent in the early 1920s (67).

Consequently, exposure to automobile emissions, including gasoline and vehicle wear, was never without concurrent exposure to Pb until its elimination in the late 20th century. The phase-out of leaded gasoline occurred in accordance with country-specific regulations with undeveloped nations often slower to implement its removal (68). This divergence in use of unleaded gasoline, alongside Pb's removal from plumbing, paint, and other sources, led to global differences in BLL.

In accordance with the results from the present study, differences in overall BLL likely gave rise to much of the global disparity in ASD prevalence (69). As mentioned above, some nations have seen higher prevalence of ASD: for example, Australia, Japan, South Korea, Sweden, and US.

With the exception of Australia, which had banned leaded gasoline in 2002, these countries completely phased-out leaded gasoline within the 1980's to early 1990's, (66,70). In contrast, countries such as Bangladesh, Ecuador, Poland, Taiwan, and Venezuela, with vastly lower rates of ASD, were slower to ban leaded gasoline. Maximum content of leaded gasoline in 1996 in these nations was 0.80, 0.50, 0.15, 0.15, and 0.85 g/l, respectively (66). Though Australia had

banned leaded gasoline after Taiwan (2000), BLL in adults in the former (1.4 ug/dl in 2010) declined more swiftly than in the latter (ranging from 2.5-4.3 ug/dl in 2010), possibly due to long-range atmospheric transport from China (71–73).

The elimination of Pb from the built environment has been a gradual process with large variations occurring across state boundaries or even between counties/municipalities. Some states had rather narrow ranges of EBLL and ASD between counties (e.g., Illinois and Pennsylvania) over the selected periods, consequently making analyses more challenging. Furthermore, it is likely that early postnatal exposures, which are not accounted for in this present study, may be sensitive to the development of ASD. Finally, states with highly fluctuating EBLL, in which Pb exposure does not follow a monotonic decline would also make analyses challenging. However, some of the differences seen in the associations between EBLL and ASD at the county/municipality level may be explained by other factors, for instance, population density and exposure to other risky modifying metals.

We observed a nonlinear association between population density and ASD, possibly indicating a major source of the metal toxicant that causes ASD. As far as we are aware, this is a unique finding. Prior studies have typically found an increased risk of ASD with urbanicity. For instance, in Denmark, Lauritsen et al. found that a higher degree of urbanization of both residence at birth and childhood were associated with a higher risk of ASD (74). A recent study from Egypt found urban residence was more associated with ASD than rural residence (75). Similarly, a study from Taiwan found greater incidence of ASD among children in satellite and urban communities than rural areas (76).

While many aspects of urbanicity could potentially be associated with ASD, air pollution has been well-studied as a contributing factor. Investigators have previously observed a link between

traffic-related air pollution and autism (77,78). A meta-analysis by Dutheil et al. found maternal exposure to particulate matter $<2.5\mu\text{m}$ (PM_{2.5}) of greatest risk (79). Third trimester exposure of PM_{2.5} has also been associated with the greatest risk (80). Nitric oxide (NO), nitrogen dioxide (NO₂), and carbon monoxide (CO), each associated with traffic, have also been implicated as risk factors (77,81,82).

The decreasing prevalence of ASD seen with larger population densities may be due to several causes. Firstly, there are numerous other pollutants the developing child may be exposed to from point sources that are commonly produced from industry in highly urbanized areas. The totality of these pollutants may have an overall mitigating effect on risk of ASD. Secondly, underdiagnosis of ASD may occur within districts of particularly high levels of the unknown toxicant if it leads to multiple neurodevelopmental disorders (e.g., attention-deficit hyperactivity disorder [ADHD]) with the potential to obscure a diagnosis of ASD, at least at an early age (83). However, the most likely explanation for the downwardly sloping curve is a consequence of higher efficiencies in transportation with increasing population density. Interestingly, in a study from Japan by Matsushashi et al., the authors found that CO₂ emissions (as measured by mileage per vehicle) were also inversely U-shaped—peaking at a population density of 100-1000 persons/km² and thereafter declining (31). Thus, fuel exhaust and non-exhaust vehicle emissions, including tire, brake wear, and road surface wear may play a role in contributing towards ASD risk (32). However, it is unlikely that ground exposure to vehicles emissions within highly urbanized areas are lower than that of less urbanized regions. Therefore, we can surmise that perinatal exposure may arise from increased vehicle emissions within the vehicle cabin, or, alternatively, the vertical structure of highly urbanized areas may decrease overall exposure to emissions.

EBLL showed the greatest magnitude of association with ASD in New Jersey and Massachusetts—the only states in which municipality-level data were applied. Although this may indicate better granularity of data, it should also be noted that these states had the highest population densities within the study. The aggregated models also showed greatest explanatory power when limited to the highest population density thresholds, particularly in conjunction with counties/municipalities of higher student counts. This may be due to higher homogeneity in environmental exposures, such as drinking water and ambient air, which in effect provides for a natural control mechanism. For instance, the heterogeneity in commuting distances is likely higher in the less urbanized counties where communities may be within commute of an urban center or completely economically independent of such cities. Low density communities may also have diverse surroundings, for instance, farmland, woodland, or the built environment, whereas urban areas would likely have a more homogenous landscape.

In the present study, potential confounders have been addressed. First, each time-series analysis was controlled for year to ensure that changes in Pb were not coincidentally aligned with other increasing or decreasing pollutants as well as to control for changes in diagnostic criteria or diagnostic fashion. Thus, any confounding environmental factors must confront the diminished likelihood of being exquisitely correlated to Pb in geography and time. Second, SES was considered in each analysis. Increased SES has often been thought to increase the likelihood of a diagnosis of ASD (84). This relationship was observed in the Massachusetts and New Jersey municipality-level analyses as well as the Illinois and Missouri county-level analyses. In contrast, after adjusting for Pb exposure and population density in the California and Ohio county-level models, we found a negative association between SES and ASD. As students with ASD are more frequently homeschooled in high SES locales, this would have the effect of

suppressing ASD prevalence. However, if we assume that the SES finding is genuine, other explanations are potentially found. For instance, more frequent maternal perinatal use of nutritional supplements among those of higher SES (84). Indeed, nutritional supplementation with or without iron has been linked to lower risk of ASD (85). Use of filtered water, potentially decreasing exposure to the unknown metal toxicant, may also contribute to decreased risk of ASD.

Due to the ecological nature of the study, it is important to address some distortions in the perception of this type of analysis among researchers. Much like all other types of studies, even randomized clinical trials, ecological studies are subject to confounding and bias. Much of the criticism surrounding ecological studies pertains to the discrepancy Robinson found between individual and ecological data (86). However, if aware of the potential biases and confounders, the ecological fallacy can be limited (87). Moreover, due to the possibility of individual-level fallacies in which population-level effects are not considered, Subramanian et al. argue for testing cross-level effects (88). It should be noted that time-variant environmental exposures, as in the current study, are less prone to the ecological fallacy as individuals maintain little control over or awareness of their personal exposures. Thus, this study, in conjunction with the previous state-level study, can be viewed as strong preliminary evidence for the role of Pb in inhibiting the onset of ASD and highlights the shortcomings of using only individual-level data.

There are several limitations to this study. First, as indicated above, the study is subject to the ecological fallacy. Second, each county/municipality had differing representation among the children undergoing measurements for BLL, making BLL less accurate in some localities than in others. In addition, locations with higher populations were likely to have more accurate ASD prevalence rates (i.e., narrower 95% confident intervals). However, regions in these

investigations are weighted similarly, independent of population. In attempt to circumvent this bias, we developed models of varying thresholds of population. Third, as proxy perinatal blood levels are local and at the population level, children who either moved away from or into any of the regions of interest could lead to a discrepancy between ASD prevalence rate and putative Pb exposure. Similarly, it is conceivable that parents of children with special needs would move away from highly populated cities and towards suburban environments. This would have the effect of downwardly biasing risk of ASD within cities and, conversely, upwardly biasing risk within the suburbs. Fourth, as mentioned earlier, only those children who were enrolled in schools and whose disability would impact their education were counted towards ASD prevalence rates. It was often the case that regions with fewer than 10 students identified with ASD were excluded from analyses due to state confidentiality requirements. The effect this may have on the study is eliminating smaller regions of potentially high Pb exposure. Finally, the changing definition of ASD over the decades and heterogeneity in applying the definitions of ASD in disparate locations are other known factors which can lead to varying levels of ASD (89,90).

Conclusion

In summary, though it has been generally acknowledged that genetic and environmental interactions are causal of ASD, knowledge of which environmental factors contributed to the condition has eluded researchers. The results from the current study offer considerable understanding of the current autism epidemic, with the assertion that as BLL has declined over the last several decades, it has left infants increasingly susceptible to ASD. This has resulted in higher absorption of another yet identified toxic metal. Though these results contrast with how Pb is typically perceived, namely, as detrimental to normal neurodevelopment, we can now

advance the notion that Pb is protective of ASD and the long-term and ongoing reduction in Pb exposure has been the primary cause of the epidemic, though not the ultimate cause of ASD. Despite the correlative nature of these studies, the abundance and scope of evidence brought forth in this study suggests that this inverse association is also causative. We must face the prospect that continuation of the policy of removing Pb from the built environment will lead to increased cases of ASD.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

Author declares no competing interests.

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Tables

Table 1. Study criteria and characteristics, by state

State	Geographic Scale	total no. of counties /municipalities in state	Minimum student count per county /municipality	Study Years*	EBLL Threshold	Obs. (n) meeting study criteria	Obs. (n) per year of study
CA	County	58	600	2013-2018	4.5 µg/dl	232	38.7
IL	County	102	600	2010-2020	10 µg/dl	286	26
MA	Municipality	351	600	2009-2020	5 µg/dl	206	17.2
MN	County	87	500	2011-2019	5 ug/dl	198	22
MO	County	114	600	2006-2018	5 µg/dl	152	11.7
NJ	Municipality	564	600	2007	10 µg/dl	59	59
OH	County	88	500	2015-2019	5 µg/dl	170	34
PA	County	67	600	2010-2018	10 µg/dl	324	36
TX	County	254	600	2019-2020	5 µg/dl	108	59
WI	County	72	600	2005-2019	5 µg/dl	375	25

* Years in which ASD prevalence was derived. New Jersey analyses totaled 3 years; however, all years were totaled into a single observation point for each municipality.

† BLL from children of various ages from period 6 years prior to student ASD counts.

EBLL: Elevated blood lead level; PPSM: Persons per square mile; N/A: Not applicable

Table 2. ASD prevalence, EBLL incidence, and population density, by state

State	ASD Prevalence				EBLL Incidence				Population Density (PPSM)	
	Mean (SD)		Range		Mean (SD)		Range		Mean (SD)	Range
	First Year	Final Year	First Year	Final Year	First Year	Final Year	First Year	Final Year	All Years	All Years
CA	1.09% (0.37%)	2.02% (1.33%)	0.44- 2.01%	0.84- 9.61%	11.6% (10.7%)	2.3% (1.6%)	1.3- 60.9%	0.0-5.9%	2827 (3978)	57 - 23,077
IL	0.74% (0.31%)	1.30% (0.37%)	0.00- 1.25%	0.35- 1.97%	3.4% (2.2%)	1.1% (0.8%)	0.3- 10.1%	0.0-3.8%	1591 (2001)	106 – 10,605
MA	1.33% (0.52%)	3.21% (1.22%)	0.69- 2.85%	1.67- 6.19%	18.7% (6.4%)	3.4% (1.4%)	8.6- 28.2%	1.7-7.1%	8407 (5639)	335 – 21,208
MN	1.21% (0.57%)	1.75% (0.88%)	0.38- 2.31%	0.50- 4.45%	8.6% (3.8%)	0.75% (0.64%)	2.4- 17.9%	0.0-2.0%	897 (1204)	41 - 4361
MO	0.72% (0.42%)	1.40% (0.46%)	0.34- 1.46%	0.66- 2.23%	36.7% (15.5%)	4.0% (3.1%)	22.0- 70.0%	1.1- 11.9%	1527 (1595)	88 - 6011
NJ	1.12% (0.46%)	N/A	0.22- 2.02%	N/A	2.2% (2.3%)	N/A	0-8.5%	N/A	7468 (9376)	515 – 51,602
OH	1.26% (0.38%)	1.76% (0.54%)	0.76- 1.96%	0.83- 3.34%	9.7% (6.8%)	4.2% (2.9%)	2.6- 38.6%	1.2- 12.0%	1136 (979)	49 - 4068
PA	1.21% (0.34%)	2.07% (0.48%)	0.42- 1.98%	1.15- 3.17%	6.1% (3.2%)	2.1% (0.8%)	2.1- 13.6%	0.4-3.6%	1799 (2648)	91 – 15,005
TX	1.59% (0.42%)	1.70% (0.49)	0.69- 2.90%	0.59- 2.76%	2.8% (1.2%)	2.4% (1.1%)	1.2-6.4%	0.7-5.6%	1015 (1026)	17 - 4017
WI	0.65% (0.29%)	1.38% (0.50%)	0.16- 1.37%	0.54- 2.50%	25.0% (10.4%)	2.9% (2.0%)	12.4- 63.2%	0.0-8.7%	963 (1230)	83 - 6018

EBLL: Elevated blood lead level; PPSM: Persons per square mile.

Table 3. Correlation of EBLL with population density and income, by state

State	EBLL vs. Population Density		EBLL vs. Income	
	Correlation (r)	P-value	Correlation (r)	P-value
CA	0.0244	0.7082	-0.0306	0.6387
IL	0.0146	0.8054	-0.1723	0.0035
MA	-0.1016	0.1463	-0.3435	<0.0001
MN	0.3188	<0.0001	0.0697	0.3291
MO	0.4282	<0.0001	-0.27101	0.0007
NJ	0.3314	0.0103	-0.6000	<0.0001
OH	0.2718	0.0003	-0.2081	0.0065
PA	0.2101	0.0001	-0.1358	0.0144
TX	-0.116	0.2553	-0.1726	0.0893
WI	0.3263	<0.0001	-0.1921	0.0002

EBLL: Elevated blood lead level.

Table 4. County/municipality-level regression models of ASD prevalence with monotonically treated population density, by state

State, Years (Minimum students per county/municipality)	Variable	EBLL only				+ adjusted for Population density				+ adjusted for Year and Income			
		Coefficient	SE	T-value	P-value	Coefficient	SE	T-value	P-value	Coefficient	SE	T-value	P-value
California, 2013-2018 (n ≥ 600)	Intercept	-3.93018	0.05456	-72.04	<0.0001	-3.96478	0.05547	-71.47	<0.0001	-4.31115	0.13576	-31.75	<0.0001
	sqrt (EBLL-5)	-1.65635	0.24977	-6.63	<0.0001	-1.71741	0.24775	-6.93	<0.0001	-0.88876	0.2871	-3.1	0.0022
	Density	-	-	-	-	1.64E-05	6.27E-06	2.62	0.0094	2.16E-05	6.93E-06	3.12	0.0021
	Year	-	-	-	-	-	-	-	-	0.08857	0.01693	5.23	<0.0001
	Income	-	-	-	-	-	-	-	-	-5E-06	2.93E-06	-1.72	0.087
	Adj. R-squared	0.1569 (P<0.0001), n = 232				0.1778 (P<0.0001), n = 232				0.2732 (P<0.0001), n = 232			
Illinois, 2010-2020 (n ≥ 600)	Intercept	0.01069	0.00047824	22.36	<0.0001	0.00611	0.00131	4.68	<0.0001	0.00203	0.00159	1.28	0.2019
	sqrt (EBLL-10)	-0.01288	0.00342	-3.77	0.0002	-0.0137	0.00335	-4.09	<0.0001	-0.0014	0.00367	-0.38	0.7027
	ln (Density)	-	-	-	-	6.83E-04	1.82E-04	3.76	0.0002	0.000362	0.000201	1.8	0.0725
	Year	-	-	-	-	-	-	-	-	0.000454	6.18E-05	7.34	<.0001
	Income	-	-	-	-	-	-	-	-	7.40E-08	5.28E-08	1.4	0.1619
	Adj. R-squared	0.0442 (P<0.0001), n = 286				0.0865 (P<0.0001), n = 286				0.2281 (P<0.0001), n = 286			
Massachusetts, 2009- 2020 (n ≥ 600)	Intercept	-3.63699	0.0431	-84.38	<0.0001	-3.5647	0.05831	-61.14	<0.0001	-4.34168	0.17429	-24.91	<0.0001
	EBLL-5	-0.00379	0.00034341	-11.04	<0.0001	-0.00385	0.00034323	-11.23	<0.0001	-0.00128	6.07E-04	-2.11	0.0365
	Density	-	-	-	-	-7.81E-06	4.27E-06	-1.83E+00	0.0689	-9.44E-06	4.11E-06	-2.29	0.0228
	Year	-	-	-	-	-	-	-	-	0.0621	0.01183	5.25	<0.0001
	Income	-	-	-	-	-	-	-	-	4.19E-06	2.08E-06	2.02E+00	0.0449
	Adj. R-squared	0.3709 (P<0.0001), n = 206				0.3781 (P<0.0001), n = 206				0.4481 (P<0.0001), n = 206			
Minnesota, 2011-2019 (n ≥ 500)	Intercept	0.09785	0.00616	15.88	<.0001	0.08878	0.00648	13.7	<.0001	0.08663	0.01373	6.31	<.0001
	ln (EBLL-5)	-0.00487	0.00171	-2.85	0.0048	-0.00599	0.00168	-3.56	0.0005	-0.00345	0.00248	-1.39	0.1663
	Density	-	-	-	-	5.82E-06	1.6E-06	3.63	0.0004	5.16E-06	1.95E-06	2.65	0.0088
	Year	-	-	-	-	-	-	-	-	0.00153	0.00108	1.41	0.1609
	Income	-	-	-	-	-	-	-	-	1.28E-07	4.86E-07	0.26	0.7929
	Adj. R-squared	0.0350 (P=0.0048), n = 198				0.914 (P<0.0001), n = 198				0.0913 (P=0.0002), n = 198			
Missouri, 2006-2018 (n ≥ 600)	Intercept	-4.17931	0.05741	-72.8	<0.0001	-3.69266	0.17036	-21.68	<0.0001	161.6535	22.30619	7.25	<.0001
	sqrt (EBLL-5)	-1.39121	0.15766	-8.82	<0.0001	-1.24275	0.1612	-7.71	<0.0001	0.80811	0.28128	2.87	0.0047
	ln (Density)	-	-	-	-	-0.07822	0.02587	-3.02	0.0029	-0.18525	0.02501	-7.41	<.0001
	Year	-	-	-	-	-	-	-	-	0.08365	0.01126	7.43	<.0001
	Income	-	-	-	-	-	-	-	-	5.11E-05	6.6E-06	7.74	<.0001
	Adj. R-squared	0.3373 (P<0.0001), n = 152				0.3715 (P<0.0001), n = 152				0.5770 (P<0.0001), n = 152			
New Jersey, 2007 (n ≥ 600)	Intercept	0.01363	7.13E-04	1.91E+01	<0.0001	0.01385	0.000756	18.33	<.0001	0.00703	0.00266	2.65	0.0106
	EBLL-10	-0.11064	0.02252	-4.91	<0.0001	-0.10346	0.02391	-4.33	<.0001	-0.06518	0.02686	-2.43	0.0185
	Density	-	-	-	-	-5.12E-08	5.65E-08	-0.91	0.3683	8.98E-10	5.71E-08	0.02	0.9875

	Income	-	-	-	-	-	-	-	-	2.27E-07	8.50E-08	2.67	0.01
	Adj. R-squared	0.2852 (P<0.0001), n = 59				0.2829 (P<0.0001), n = 59				0.3535 (P<0.0001), n = 59			
Ohio, 2015-2019 (n ≥ 500)	Intercept	-4.38893	0.11162	-39.32	<0.0001	-4.51313	0.12413	-36.36	<0.0001	-4.20389	0.15881	-26.47	<.0001
	ln (EBLL-5)	-0.04516	0.03672	-1.23	0.2205	-0.06533	0.03747	-1.74	0.0831	-0.04065	0.04565	-0.89	0.3745
	Density	-	-	-	-	5.67E-05	2.59E-05	2.19	0.0301	8.16E-05	2.65E-05	3.08	0.0024
	Year	-	-	-	-	-	-	-	-	0.07209	0.01937	3.72	0.0003
	Income	-	-	-	-	-	-	-	-	-1.9E-05	6.7E-06	-2.82	0.0055
	Adj. R-squared	0.003 (P=0.2205), n = 170				0.025 (P=0.0447), n = 170				0.1707 (P<0.0001), n = 170			
Pennsylvania, 2010-2018 (n ≥ 600)	Intercept	0.14148	0.00308	45.99	<0.0001	0.11416	0.00668	17.1	<0.0001	0.0784	0.00792	9.89	<.0001
	sqrt (EBLL-10)	-0.09611	0.01661	-5.79	<0.0001	-0.11019	0.01641	-6.72	<0.0001	-0.00352	0.01873	-0.19	0.851
	ln (Density)	-	-	-	-	0.00429	0.000938	4.57	<0.0001	0.01215	0.02301	0.53	0.5977
	Year	-	-	-	-	-	-	-	-	0.00326	0.00093	3.5	0.0005
	Income	-	-	-	-	-	-	-	-	0.00342	0.000347	9.87	<.0001
	Adj. R-squared	0.0914 (P<0.0001), n = 324				0.1443 (P<0.0001), n = 324				0.3406 (P<0.0001), n = 324			
Texas, 2019-2020 (n ≥ 600)	Intercept	0.01954	0.00103	18.94	<.0001	0.01857	0.00116	15.98	<.0001	0.01604	0.00292	5.49	<.0001
	EBLL-5	-0.00116	0.000357	-3.26	0.0015	-0.00108	0.000357	-3.03	0.0031	-0.00102	0.000372	-2.75	0.007
	sqrt (Density)	-	-	-	-	6.97E-07	4.01E-07	1.74	0.0849	2.3E-05	2.85E-05	0.81	0.4205
	Year	-	-	-	-	-	-	-	-	0.000712	0.000872	0.82	0.4162
	Income	-	-	-	-	-	-	-	-	5.61E-08	8.72E-08	0.64	0.5219
	Adj. R-squared	0.0823 (P=0.0022), n = 108				0.0995 (P=0.0011), n = 108				0.0750 (P=0.0018), n = 108			
Wisconsin, 2005-2020 (n ≥ 600)	Intercept	0.12019	0.00262	45.91	<.0001	0.09474	0.00562	16.86	<.0001	0.07266	0.01183	6.14	<.0001
	sqrt (EBLL-5)	-0.0568	0.0074	-7.68	<.0001	-0.06212	0.00724	-8.58	<.0001	-0.02149	0.01484	-1.45	0.1483
	ln (Density)	-	-	-	-	0.00434	0.000855	5.08	<.0001	0.00329	0.000932	3.53	0.0005
	Year	-	-	-	-	-	-	-	-	0.00142	0.000423	3.34	0.0009
	Income	-	-	-	-	-	-	-	-	1.50E-07	2.55E-07	0.59	0.5573
	Adj. R-squared	0.1341 (P<0.0001), n = 375				0.188 (P<0.0001), n = 375				0.2090 (P<0.0001), n = 375			

Model dependent variables: California: natural log of ASD prevalence; Illinois: ASD prevalence; Massachusetts: natural log of ASD prevalence; Minnesota: square root of ASD prevalence; Missouri: natural log of ASD prevalence; New Jersey: ASD prevalence; Ohio: natural log of ASD prevalence; Pennsylvania: square root of ASD prevalence; Texas: ASD prevalence; Wisconsin: square root of ASD prevalence.

EBLL-5: Annual elevated blood level ≥5 µg/dl; EBLL-10: Annual elevated blood level ≥5 µg/dl; Density: Population density (persons per square mile); Year: Year of derived ASD prevalence.

†New Jersey ASD prevalence is limited to only 1 year, therefore adjustment by year was not evaluated in regression.

Table 5. County/municipality-level regression models of ASD prevalence with quadratically treated population density, by state

State (minimum students per county/municipality)	Variable	Quadratic Model				+ adjusted for Year and Income			
		Coefficient	SE	T-value	P-value	Coefficient	SE	T-value	P-value
California, 2013-2018 (n ≥ 600)	Intercept	-3.9895	0.05996	-66.53	<0.0001	-4.32024	0.13542	-31.9	<0.0001
	sqrt (EBLL-5)	-1.72948	0.2479	-6.98	<0.0001	-0.91335	0.28653	-3.19	0.0016
	Density	3.31E-05	1.66E-05	1.99	0.0474	4.63E-05	1.69E-05	2.74	0.0067
	Density*Density	-8.38E-10	7.74E-10	-1.08	0.2798	-1.18E-09	7.40E-10	-1.6	0.111
	Year	-	-	-	-	0.08847	0.01687	5.24	<0.0001
	Income	-	-	-	-	-6E-06	2.98E-06	-2.01	0.0458
	Adj. R-squared	0.1784 (P<0.0001), n = 232				0.2781 (P<0.0001), n = 232			
Illinois, 2010-2020 (n ≥ 600)	Intercept	0.00946	0.000547	17.31	<0.0001	0.00518	0.00177	2.93	0.0037
	sqrt (EBLL-10)	-0.01324	0.00332	-3.99	<0.0001	-0.00242	0.00364	-0.67	0.5063
	Density	1.15E-06	2.92E-07	3.94	0.0001	8.79E-07	3.44E-07	2.55	0.0112
	Density*Density	-8.51E-11	2.74E-11	-3.11	0.0021	-6.27E-11	3.04E-11	-2.06	0.0399
	Year	-	-	-	-	0.000445	6.11E-05	7.29	<.0001
	Income	-	-	-	-	2.15E-08	5.73E-08	0.38	0.7075
	Adj. R-squared	0.0992 (P<0.0001), n = 286				0.2399 (P<0.0001), n = 286			
Massachusetts, 2009-2020 (n ≥ 600)	Intercept	-3.54806	0.08014	-44.27	<0.0001	-4.28195	0.18112	-23.64	<0.0001
	EBLL-5	-0.00385	3.45E-04	-1.12E+01	<0.0001	-0.00121	6.09E-04	-1.98E+00	0.0488
	Density	-1.26E-05	1.65E-05	-7.70E-01	0.4435	-2.79E-05	1.60E-05	-1.75E+00	0.0823
	Density*Density	2.27E-10	7.46E-10	3.00E-01	0.7618	8.79E-10	7.34E-10	1.20E+00	0.2329
	Year	-	-	-	-	0.06407	0.01193	5.37	<0.0001
	Income	-	-	-	-	3.73E-06	2.11E-06	1.77E+00	0.0782
	Adj. R-squared	0.3753 (P<0.0001), n = 206				0.4493 (P<0.0001), n = 206			
Minnesota, 2011-2019 (n ≥ 500)	Intercept	0.08598	0.00654	13.15	<.0001	0.09943	0.01438	6.91	<.0001
	ln (EBLL-5)	-0.00558	0.00168	-3.33	0.001	-0.00302	0.00245	-1.23	0.2202
	Density	1.77E-05	5.58E-06	3.17	0.0018	2.24E-05	6.84E-06	3.27	0.0013
	Density*Density	-2.86E-09	1.29E-09	-2.22	0.0276	-3.82E-09	1.46E-09	-2.62	0.0095
	Year	-	-	-	-	0.00171	0.00107	1.6	0.1123
	Income	-	-	-	-	-5.25E-07	5.40E-07	-0.97	0.3317
	Adj. R-squared	0.1093 (P<0.0001), n = 198				0.1180 (P<0.0001), n = 198			
Missouri, 2006-2018 (n ≥ 600)	Intercept	-4.18183	0.07221	-57.91	<0.0001	177.6877	25.88966	6.86	<.0001
	EBLL-5	-1.25776	0.17328	-7.26	<0.0001	0.94085	0.32975	2.85	0.005
	Density	-2E-05	5.48E-05	-0.36	0.7204	-0.00013	5.91E-05	-2.12	0.0358
	Density*Density	-2.23E-09	8.95E-09	-0.25	0.8039	3.80E-09	9.62E-09	0.39	0.6936
	Year	-	-	-	-	0.09216	0.01308	7.04	<.0001
	Income	-	-	-	-	4.3E-05	7.37E-06	5.83	<.0001
	Adj. R-squared	0.3437 (P<0.0001), n = 152				0.5272 (P<0.0001), n = 152			
New Jersey, 2007 (n ≥ 600)	Intercept	0.01335	0.000873	15.29	<.0001	0.00644	0.00269	2.4	0.02
	EBLL-10	-0.12381	0.02985	-4.15	0.0001	-0.08612	0.03151	-2.73	0.0085

Ohio, 2015-2019 (n ≥ 500)	Density	1.62E-07	1.96E-07	0.82	0.413	2.25E-07	1.87E-07	1.2	0.2354
	Density*Density	-4.33E-12	3.81E-12	-1.13	0.2618	-4.53E-12	3.61E-12	-1.25	0.2152
	Income	-	-	-	-	2.29E-07	8.46E-08	2.71	0.0091
	Adj. R-squared	0.2866 (P<0.0001), n = 59					0.3602 (P<0.0001), n = 59		
	Intercept	-4.39879	0.10995	-40.01	<0.0001	-4.20981	0.14306	-29.43	<.0001
	ln (EBLL-5)	-0.11087	0.03345	-3.31	0.0011	-0.07737	0.04154	-1.86	0.0643
	Density	-0.00044	7.33E-05	-6.04	<0.0001	-0.00035	7.35E-05	-4.82	<.0001
	Density*Density	1.42E-07	1.97E-08	7.17	<0.0001	1.21E-07	1.93E-08	6.27	<.0001
	Year	-	-	-	-	0.05828	0.01759	3.31	0.0011
	Income	-	-	-	-	-1.3E-05	6.12E-06	-2.05	0.0419
Pennsylvania, 2010-2018 (n ≥ 600)	Adj. R-squared	0.2512 (P<0.0001), n = 170					0.3270 (P<0.0001), n = 170		
	Intercept	0.13784	0.0031	44.49	<0.0001	0.09835	0.00734	13.4	<.0001
	sqrt (EBLL-10)	-0.11192	0.0161	-6.95	<0.0001	-0.00851	0.01802	-0.47	0.6372
	Density	4.87E-06	1.05E-06	4.66	<0.0001	0.01789	0.02249	0.8	0.427
	Density*Density	-2.28E-10	7.11E-11	-3.2	0.0015	4.64E-06	1.08E-06	4.3	<.0001
	Year	-	-	-	-	-2.35E-10	7.32E-11	-3.21	0.0015
	Income	-	-	-	-	0.00338	0.000338	10.02	<.0001
	Adj. R-squared	0.1758 (P<0.0001), n = 324					0.3705 (P<0.0001), n = 324		
	Intercept	0.01943	0.00119	16.33	<.0001	0.01705	0.00284	5.99	<.0001
	EBLL-5	-0.001	0.00035	-2.87	0.005	-0.00092	0.000362	-2.54	0.0126
Texas, 2019-2020 (n ≥ 600)	Density	-2.1E-06	1.23E-06	-1.73	0.0874	-2.2E-06	1.25E-06	-1.75	0.0831
	Density*Density	8.61E-10	3.55E-10	2.42	0.0172	8.67E-10	3.58E-10	2.42	0.0172
	Year	-	-	-	-	0.000722	0.000845	0.85	0.3949
	Income	-	-	-	-	4.52E-08	8.44E-08	0.54	0.5932
	Adj. R-squared	0.1394 (P=0.0008), n = 108					0.1310 (P=0.0011), n = 108		
	Intercept	0.11694	0.00286	40.89	<.0001	0.0863	0.01267	6.81	<.0001
	sqrt (EBLL-5)	-0.06396	0.00746	-8.57	<.0001	-0.01894	0.01548	-1.22	0.222
	Density	7.64E-06	2.1E-06	3.63	0.0003	7.24E-06	2.15E-06	3.37	0.0008
	Density*Density	-7.14E-10	3.69E-10	-1.93	0.0541	-8.79E-10	3.77E-10	-2.33	0.0202
	Year	-	-	-	-	0.00149	0.000434	3.43	0.0007
Wisconsin, 2005-2020 (n ≥ 600)	Income	-	-	-	-	1.76E-07	2.51E-07	0.7	0.4843
	Adj. R-squared	0.1913 (P<0.0001), n = 375					0.2130 (P<0.0001), n = 375		
	Intercept	0.11694	0.00286	40.89	<.0001	0.0863	0.01267	6.81	<.0001
	sqrt (EBLL-5)	-0.06396	0.00746	-8.57	<.0001	-0.01894	0.01548	-1.22	0.222
	Density	7.64E-06	2.1E-06	3.63	0.0003	7.24E-06	2.15E-06	3.37	0.0008
	Density*Density	-7.14E-10	3.69E-10	-1.93	0.0541	-8.79E-10	3.77E-10	-2.33	0.0202
	Year	-	-	-	-	0.00149	0.000434	3.43	0.0007
	Income	-	-	-	-	1.76E-07	2.51E-07	0.7	0.4843
	Adj. R-squared	0.1913 (P<0.0001), n = 375					0.2130 (P<0.0001), n = 375		
	Intercept	0.11694	0.00286	40.89	<.0001	0.0863	0.01267	6.81	<.0001

Model dependent variables: California: square root of ASD prevalence; Illinois: ASD prevalence; Massachusetts: natural log of ASD prevalence; Minnesota: square root of ASD prevalence; Missouri: natural log of ASD prevalence; New Jersey: ASD prevalence; Ohio: natural log of ASD prevalence; Pennsylvania: square root of ASD prevalence; Texas: ASD prevalence; Wisconsin: square root of ASD prevalence. EBLL-5: Annual elevated blood level ≥5 µg/dl; EBLL-10: Annual elevated blood level ≥5 µg/dl; Density: Population density (persons per square mile); Year: Year of derived ASD prevalence.

†New Jersey ASD prevalence is limited to only 1 year, therefore adjustment by year was not evaluated in regression.

Table 6. Aggregated multi-state analyses: model criteria and characteristics

Model #	Model Criteria				Model Characteristics						
	Geography	Years	Pop. Density (PPSM)	Total Student Count	Total Obs. (n)	Total Counties /Municipalities (n)	Total States (n)	ASD Prevalence		EBLL-5 Incidence	
								Mean (SD)	Range	Mean (SD)	Range
1	County/Municipality	Any	Any	≥600	1564	485	8	1.37% (0.68%)	0.15-9.61%	8.73% (8.49%)	0.00-69.96%
2	County-only*	Any	Any	≥600	1348	464	7	1.28% (0.60%)	0.15-9.61%	8.47% (8.66%)	0.00-69.96%
3	County/Municipality	Any	≥2000	≥600	466	104	8	1.59% (0.70%)	0.34-6.19%	9.94% (10.38%)	0.00-69.96%
4	County/Municipality	Any	≥4000	≥600	257	43	7	1.77% (0.80%)	0.34-6.19%	11.97% (11.64%)	0.85-69.96%
5	County/Municipality	Any	≥6000	≥600	178	33	5	1.73% (0.83%)	0.34-6.19%	13.02% (12.79)	1.01-69.96%
6	County/Municipality	Any	≥6000	≥1000	123	27	5	1.61% (0.80%)	0.34-4.59%	14.91% (14.53%)	1.01-69.96%
7	County/Municipality	Any	≥6000	≥2000	71	23	5	1.45% (0.66%)	0.34-2.88%	17.19% (17.73%)	1.01-69.96%
8	County/Municipality	2005-2013	Any	≥600	486	313	5	1.08% (0.45%)	0.16-3.05%	16.16% (10.25%)	1.30-69.96%
9	County/Municipality	2014-2020	Any	≥600	1068	363	8	1.50% (0.72%)	0.15-9.61%	5.37% (4.59%)	0.00-39.28%

*The county-only model was fitted without the addition of Massachusetts municipalities.
EBLL-5: Elevated blood level ≥5 µg/dl; PPSM: Persons per square mile.

Table 7. Aggregated multistate Model #1: characteristics by year

Year*	Observations (n)	States (n)	ASD Prevalence		EBLL-5 Incidence		Population Density	
			Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
2005	26	1	0.65% (0.29%)	0.16-1.37%	25.68% (10.50%)	12.35-63.24%	932 (1241)	83-6018
2006	33	2	0.80% (0.30%)	0.23-1.48%	26.62% (12.74%)	9.27-69.96%	1200 (1457)	83-6018
2007	35	2	0.86% (0.36%)	0.29-1.89%	23.49% (11.69%)	7.25-67.90%	1192 (1423)	83-6018
2008	36	2	0.99% (0.30%)	0.47-1.57%	22.17% (10.69%)	10.50-59.05%	1147 (1414)	83-6018
2009	53	3	1.06% (0.45%)	0.26-2.85%	19.20% (9.43%)	6.84-50.60%	3016 (4417)	83-21,208
2010	54	3	1.10% (0.44%)	0.36-2.59%	16.60% (7.81%)	4.56-35.09%	3509 (4865)	83-21,208
2011	72	4	1.19% (0.43%)	0.41-2.41%	12.89% (6.22%)	2.43-33.48%	2531 (4108)	18-21,208
2012	71	4	1.22% (0.46%)	0.48-2.44%	11.04% (5.58%)	3.62-31.43%	2801 (4439)	18-21,208
2013	106	5	1.19% (0.47%)	0.30-3.05%	10.17% (8.28%)	1.30-60.90%	2976 (4362)	26-23,077
2014	139	6	1.22% (0.50%)	0.15-3.85%	7.72% (4.76%)	0.90-29.89%	2701 (4193)	18-23,077
2015	169	7	1.30% (0.53%)	0.44-4.01%	7.54% (5.50%)	0.00-38.56%	2399 (3830)	18-23,077
2016	170	7	1.45% (0.67%)	0.32-6.09%	5.78% (4.77%)	0.00-33.62%	2283 (3623)	18-23,077
2017	165	7	1.54% (0.72%)	0.30-6.94%	4.70% (3.91%)	0.00-24.49%	2178 (3404)	18-23,077
2018	173	7	1.65% (0.86%)	0.32-9.61%	4.55% (4.64%)	0.00-39.28%	2288 (3638)	18-23,077
2019	165	6	1.63% (0.68%)	0.33-4.81%	3.53% (2.72%)	0.00-19.00%	2136 (3167)	17-21,208
2020	97	3	1.83% (0.88%)	0.35-6.19%	3.28% (2.13%)	0.72-11.50%	2357 (3626)	17-21,208

*EBLL-5 is sourced from 6 years prior to year indicated.
EBLL-5: Elevated blood level ≥ 5 $\mu\text{g/dl}$.

Table 8. Aggregated multistate regression models of ln (ASD)

Model #	Variable	Model: EBL-5				Model: + adjusted for Population Density				Model: + adjusted for Year and Income			
		Coefficient	SE	T-Value	P-Value	Coefficient	SE	T-Value	P-Value	Coefficient	SE	T-Value	P-Value
#1, n = 1564	Intercept	-3.99595	0.0248	-161.15	<.0001	-4.08621	0.02413	-169.36	<.0001	-4.67528	0.10465	-44.67	<.0001
	sqrt (EBLL-5)	-1.48781	0.08394	-17.73	<.0001	-1.66022	0.07961	-20.86	<.0001	-1.03408	0.12191	-8.48	<.0001
	Density	-	-	-	-	8.23E-05	7.11E-06	11.57	<.0001	7.26E-05	7.18E-06	10.12	<.0001
	Density*Density	-	-	-	-	-2.91E-09	4.03E-10	-7.23	<.0001	-2.48E-09	4.01E-10	-6.19	<.0001
	Year	-	-	-	-	-	-	-	-	0.02779	0.00397	7	<.0001
	Income	-	-	-	-	-	-	-	-	9.20E-07	1.49E-06	0.62	0.5369
	Adjusted R-Square (P-value)	0.1669 (P < 0.0001)				0.2691 (P < 0.0001)				0.2914 (P < 0.0001)			
#2, n = 1358	Intercept	-4.04428	0.02403	-168.32	<.0001	-4.09111	0.02486	-164.57	<.0001	-4.55058	0.11563	-39.35	<.0001
	sqrt (EBLL-5)	-1.54589	0.08255	-18.73	<.0001	-1.60816	0.08192	-19.63	<.0001	-1.07139	0.12409	-8.63	<.0001
	Density	-	-	-	-	5.2E-05	9.3E-06	5.6	<.0001	4.3E-05	9.64E-06	4.46	<.0001
	Density*Density	-	-	-	-	-1.59E-09	5.40E-10	-2.95	0.0032	-1.17E-09	5.37E-10	-2.17	0.0302
	Year	-	-	-	-	-	-	-	-	0.02519	0.00409	6.16	<.0001
	Income	-	-	-	-	-	-	-	-	-1.6E-06	2.01E-06	-0.8	0.4246
	Adjusted R-Square (P-value)	0.2049 (P < 0.0001)				0.2288 (P < 0.0001)				0.2531 (P < 0.0001)			
#3, n = 466	Intercept	-3.778	0.0402	-93.98	<.0001	-4.10174	0.05165	-79.42	<.0001	-4.9884	0.18203	-27.4	<.0001
	sqrt (EBLL-5)	-1.59865	0.12751	-12.54	<.0001	-1.81135	0.11984	-15.11	<.0001	-0.98205	0.19919	-4.93	<.0001
	Density	-	-	-	-	9.87E-05	1.24E-05	7.98	<.0001	9.04E-05	1.23E-05	7.36	<.0001
	Density * Density	-	-	-	-	-3.62E-09	5.65E-10	-6.4	<.0001	-3.37E-09	5.67E-10	-5.94	<.0001
	Year	-	-	-	-	-	-	-	-	0.03772	0.00748	5.04	<.0001
	Income	-	-	-	-	-	-	-	-	4.17E-06	1.75E-06	2.38	0.0175
	Adjusted R-Square (P-value)	0.2514 (P < 0.0001)				0.3691 (P < 0.0001)				0.4088 (P < 0.0001)			
#4, n = 257	Intercept	-3.39636	0.05087	-66.77	<.0001	-3.36669	0.06736	-49.98	<.0001	-4.17913	0.26703	-15.65	<.0001
	sqrt (EBLL-5)	-2.33568	0.14703	-15.89	<.0001	-2.35024	0.14877	-15.8	<.0001	-1.62104	0.27328	-5.93	<.0001
	Density	-	-	-	-	-2.8E-06	4.11E-06	-0.67	0.5017	-1.2E-06	4.19E-06	-0.29	0.7747
	Year	-	-	-	-	-	-	-	-	0.03676	0.01077	3.41	0.0008
	Income	-	-	-	-	-	-	-	-	1.03E-06	1.9E-06	0.54	0.5885
	Adjusted R-Square (P-value)	0.4954 (P < 0.0001)				0.4943 (P < 0.0001)				0.5138 (P < 0.0001)			
#5, n = 178	Intercept	-3.3188	0.05954	-55.74	<0.0001	-3.23736	0.09252	-34.99	<0.0001	-3.46302	0.32134	-10.78	<.0001
	sqrt (EBLL-5)	-2.58491	0.165	-15.67	<0.0001	-2.64765	0.17366	-15.25	<0.0001	-2.44356	0.32717	-7.47	<.0001
	Density	-	-	-	-	-5.60E-06	4.87E-06	-1.15	0.2521	-5.2E-06	5.81E-06	-0.9	0.3689
	Year	-	-	-	-	-	-	-	-	0.00955	0.01298	0.74	0.4628
	Income	-	-	-	-	-	-	-	-	5.75E-07	2.7E-06	0.21	0.8315
	Adjusted R-Square (P-value)	0.58 (P < 0.0001)				0.5808 (P < 0.0001)				0.5773 (P < 0.0001)			
#6, n = 123	Intercept	-3.34334	0.07044	-47.47	<0.0001	-3.30098	0.10766	-30.66	<0.0001	-3.78468	0.3737	-10.13	<.0001
	sqrt (EBLL-5)	-2.58702	0.18244	-14.18	<0.0001	-2.61913	0.19307	-13.57	<0.0001	-2.16357	0.35673	-6.06	<.0001
	Density	-	-	-	-	-3.00E-06	5.76E-06	-0.52	0.603	1.01E-06	6.83E-06	0.15	0.8827
	Year	-	-	-	-	-	-	-	-	0.02427	0.01531	1.58	0.1157
	Income	-	-	-	-	-	-	-	-	-2.4E-06	4.7E-06	-0.52	0.6054
	Adjusted R-Square (P-value)	0.6212 (P < 0.0001)				0.6189 (P < 0.0001)				0.6218 (P < 0.0001)			
#7, n = 71	Intercept	-3.471	0.07247	-47.89	<0.0001	-3.65216	0.10891	-33.53	<0.0001	-3.27824	0.48839	-6.71	<.0001
	sqrt (EBLL-5)	-2.37995	0.1748	-13.62	<0.0001	-2.22841	0.18381	-12.12	<0.0001	-2.49341	0.39476	-6.32	<.0001

	Density	-	-	-	-	1.14E-05	5.24E-06	2.18	0.0324	1.36E-05	7.06E-06	1.92	0.0594
	Year	-	-	-	-	-	-	-	-	-0.01179	0.01768	-0.67	0.5072
	Income	-	-	-	-	-	-	-	-	-4.3E-06	7.18E-06	-0.6	0.5485
	Adjusted <i>R</i> -Square (P-value)	0.7248 (P < 0.0001)				0.7391 (P < 0.0001)				0.7337 (P < 0.0001)			
#8, n = 486	Intercept	-4.1885	0.06406	-65.39	<.0001	-4.21674	0.06068	-69.49	<.0001	-5.27553	0.20061	-26.3	<.0001
	EBLL-5	-1.12405	0.15932	-7.06	<.0001	-1.37254	0.15514	-8.85	<.0001	-0.53127	0.21889	-2.43	0.0156
	Density	-	-	-	-	7.27E-05	1.25E-05	5.83	<.0001	5.27E-05	1.29E-05	4.08	<.0001
	Density * Density	-	-	-	-	-2.56E-09	7.18E-10	-3.57	0.0004	-2.12E-09	7.19E-10	-2.95	0.0033
	Year	-	-	-	-	-	-	-	-	0.04159	0.00962	4.32	<.0001
	Income	-	-	-	-	-	-	-	-	1.24E-05	2.72E-06	4.55	<.0001
	Adjusted <i>R</i> -Square (P-value)	0.0914 (P < 0.0001)				0.1876 (P < 0.0001)				0.2343 (P < 0.0001)			
#9, n = 1078	Intercept	-4.02052	0.03332	-120.67	<.0001	-4.10382	0.03205	-128.05	<.0001	-4.77443	0.14786	-32.29	<.0001
	sqrt (EBLL-5)	-1.26223	0.14371	-8.78	<.0001	-1.51479	0.13652	-11.1	<.0001	-1.24322	0.14904	-8.34	<.0001
	Density	-	-	-	-	8.33E-05	8.72E-06	9.56	<.0001	8.4E-05	8.63E-06	9.72	<.0001
	Density * Density	-	-	-	-	-2.89E-09	4.89E-10	-5.91	<.0001	-2.75E-09	4.79E-10	-5.75	<.0001
	Year	-	-	-	-	-	-	-	-	0.04245	0.0068	6.24	<.0001
	Income	-	-	-	-	-	-	-	-	-3.9E-06	1.76E-06	-2.2	0.0279
	Adjusted <i>R</i> -Square (P-value)	0.066 (P < 0.0001)				0.1802 (P < 0.0001)				0.2151 (P < 0.0001)			

Model criteria are found in Table 5. All models were fitted with dependent variable of natural log of percentage of children aged 6 years with autism spectrum disorder (ASD). EBLL-5: Elevated blood level ≥ 5 $\mu\text{g/dl}$; Density: Population density (persons per square mile); Year: Year of derived ASD prevalence; ln: natural log; sqrt: square root.

Figures

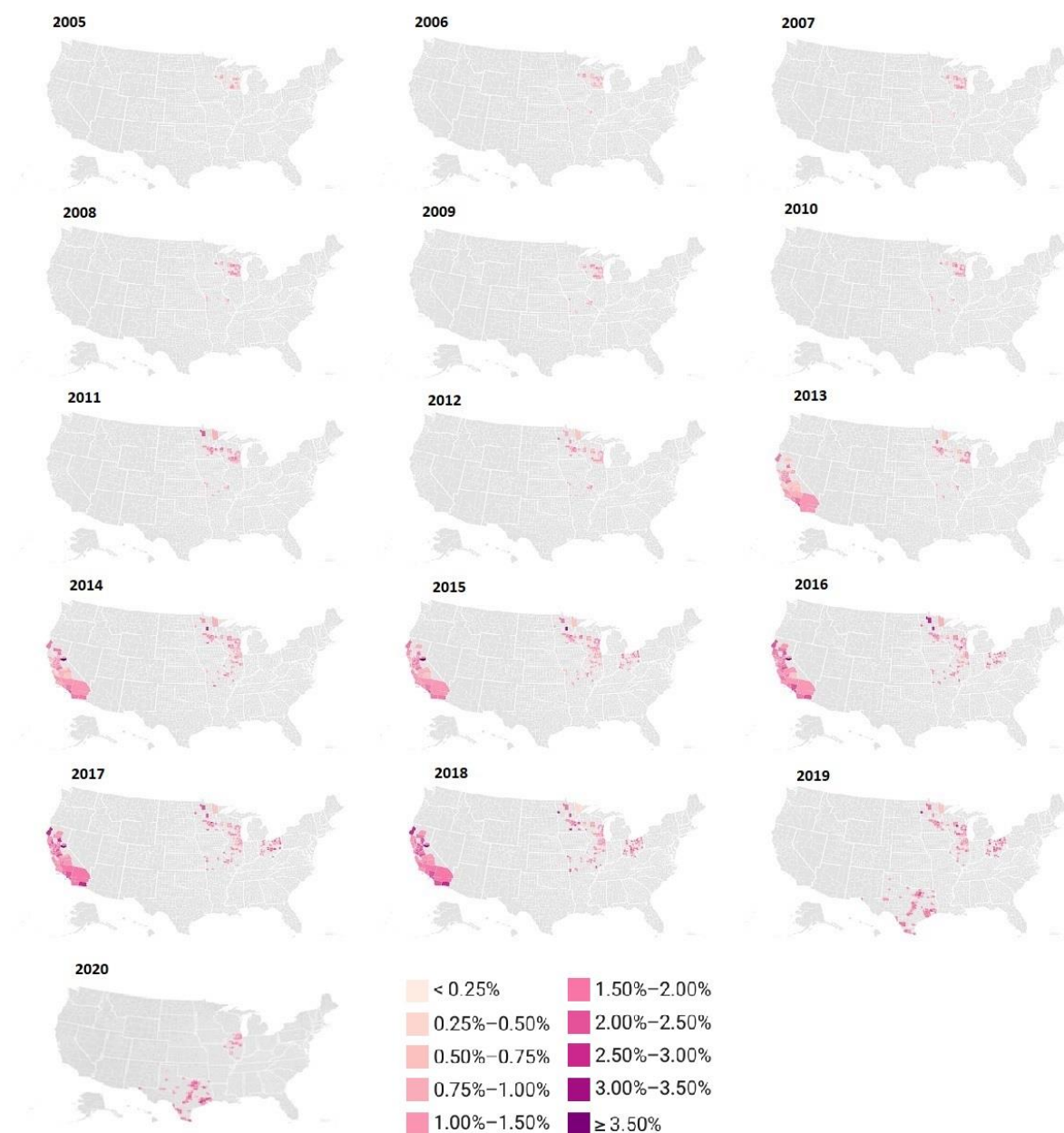


Figure 1. Choropleth of ASD prevalence by year in aggregated county Model #2 (conducted without the municipalities of Massachusetts).

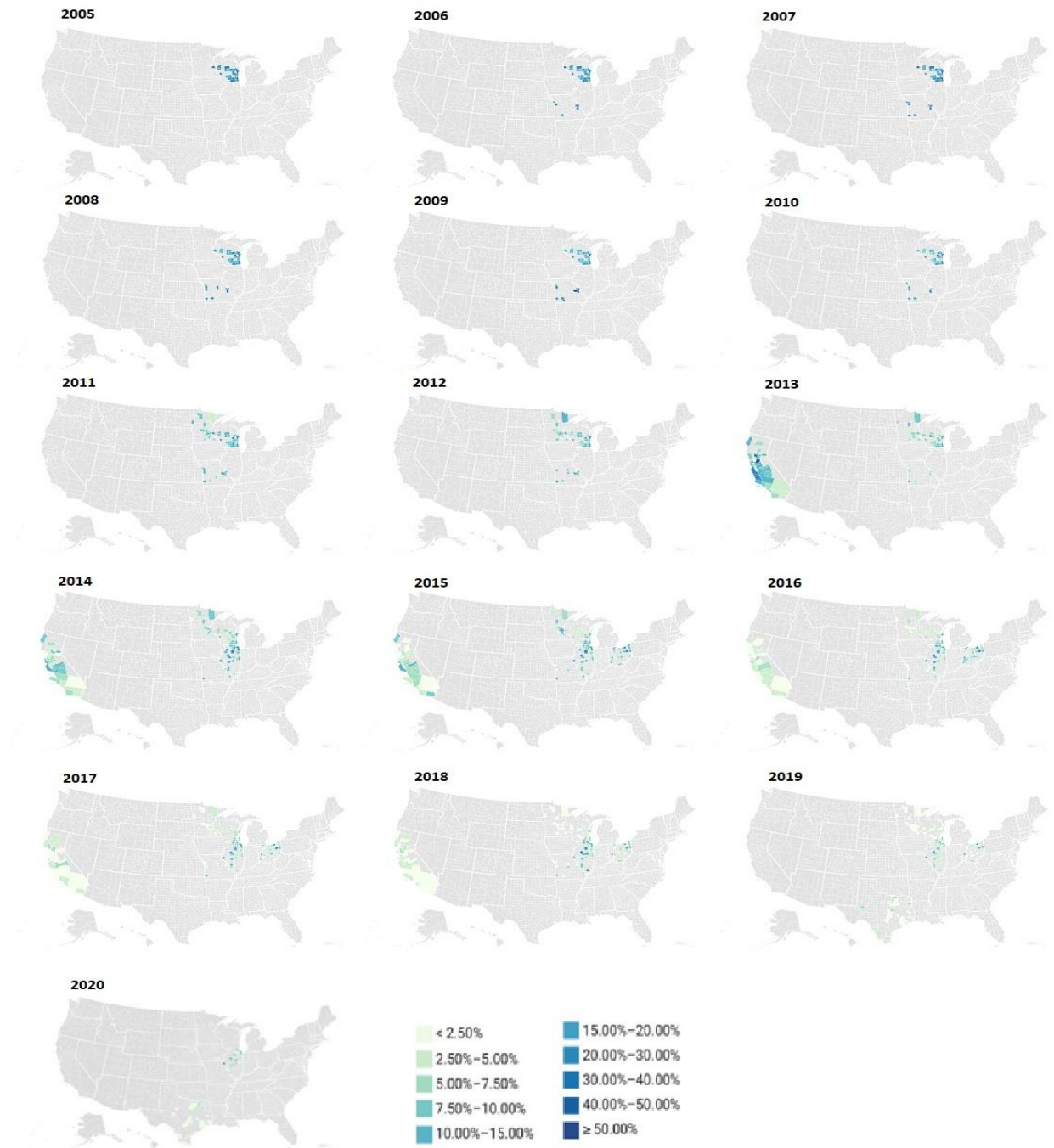


Figure 2. Choropleth of annual elevated blood lead level ≥ 5 $\mu\text{g}/\text{dl}$ (EBLL-5) incidence by year in the aggregated county Model #2 (conducted without the municipalities of Massachusetts).



Figure 3. Scatterplots of aggregated county/municipality-level time-series study of 8 states: Observations included municipalities with ≥ 600 students aged 6 years or in 1st grade ($n=1599$). In aggregate, observations were confined to a period from 2005-2006 through 2020-2021 school years. A) ASD prevalence vs. EBL-5 by state; B) ASD prevalence vs. population density (persons per square mile). EBL-5: annual elevated blood lead level ≥ 5 $\mu\text{g/dl}$ of children 6 years prior to ASD prevalence.