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Integrated Science Assessment for Lead

Appendix 6: Immune System Effects

External Review Draft

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Health and Environmental Effects Assessment Division
Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

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DOCUMENT GUIDE

This Document Guide is intended to orient readers to the organization of the Lead (Pb) Integrated Science Assessment (ISA) in its entirety and to the sub-section of the ISA at hand (indicated in bold). The ISA consists of the Front Matter (list of authors, contributors, reviewers, and acronyms), Executive Summary, Integrated Synthesis, and 12 appendices, which can all be found at https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282.

Front Matter

Executive Summary

Integrative Synthesis

Appendix 1. Lead Source to Concentration

Appendix 2. Exposure, Toxicokinetics, and Biomarkers

Appendix 3. Nervous System Effects

Appendix 4. Cardiovascular Effects

Appendix 5. Renal Effects

Appendix 6. Immune System Effects

Appendix 7. Hematological Effects

Appendix 8. Reproductive and Developmental Effects

Appendix 9. Effects on Other Organ Systems and Mortality

Appendix 10. Cancer

Appendix 11. Effects of Lead in Terrestrial and Aquatic Ecosystems

Appendix 12. Process for Developing the Pb Integrated Science Assessment

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

AQCD	Air Quality Criteria for Lead
anti-TT	anti-tetanus toxoid
BLL	blood lead level
BMI	body mass index
BW	body weight
Cd	cadmium
CD	cluster of differentiation
CI	confidence interval
CMI	cell-mediated immune
Con A	Concanavalin A
CR1	complement receptor type 1
d	day, days
DNFB	1-Fluoro-2,4-dinitrobenzene
DTH	delayed-type hypersensitivity
e-waste	electronic-waste
EDEN	Effect of Diet and Exercise on Immunotherapy and the Microbiome
EEs	effects estimates
EGFP	enhanced green fluorescent protein
ELISA	enzyme-linked immunosorbent assay
F	female
Fe	iron
GFAAS	graphite furnace atomic absorption spectrometry
GM-CSF	granulocyte-macrophage colony-stimulating factor
h	hour, hours
HBc	Hepatitis B core
HBsAb	Hepatitis B surface antigen
HBV	Hepatitis B virus
Hib	Haemophilus influenzae type B
HLA-DR	Major histocompatibility complex (MHC) II cell surface receptor
HR	hazard ratio
ICR	Institute for Cancer Research
ICP-MS	inductively coupled plasma mass spectrometry
IFN-γ	interferon-gamma
Ig-	immunoglobulin type –
IL-	interleukin type –
ILC	innate lymphoid cells
ILCP	innate lymphoid cell progenitor
ISA	Integrated Science Assessment
ISO	isolation
KNHANES	Korea National Health and Nutrition Examination Survey
ln	natural logarithm
M	male
MMR	measles, mumps, and rubella
M/F	male/female
min	minute
mo	month
MRSA	methicillin-resistant Staphylococcus aureus
MSSA	methicillin-sensitive Staphylococcus aureus
NHANES	National Health and Nutrition Examination Survey
NK	natural killer
NO	nitric oxygen
NR	not reported
OR	odds ratio
Pb	lead
PbO NPs	lead oxide nanoparticles
PCR	polymerase chain reaction
PECOS	Population, Exposure, Comparison, Outcome, and Study Design
PND	postnatal day
ppm	parts per million
Q	quartile
ROS	reactive oxygen species
RR	risk ratio
RSV	respiratory syncytial virus
S/CO	signal to cut-off
SCORAD	scoring atopic dermatitis
SD	standard deviation
SES	socioeconomic status
SPT	skin prick test
STELLAR	Systemic Tracking of Elevated Lead Levels and Remediation
T	tertile
TDAR	T cell dependent antibody response
Th2	T cell-derived helper cell 2
TNF	tumor necrosis factor
Treg	regulatory T cells
TSLP	thymic stromal lymphopoietin
TT	tetanus toxoid
tTG	tissue transglutaminase
WBC	white blood cell
wk	week, weeks
yr	year, years
vs.	versus

APPENDIX 6 IMMUNE SYSTEM EFFECTS

Causality Determinations for Pb Exposure and Immune System Effects

This appendix characterizes the scientific evidence that supports causality determinations for lead (Pb) exposure and immune system effects. The types of studies evaluated within this appendix are consistent with the overall scope of the ISA as detailed in the Process Appendix (see Section 12.4). In assessing the overall evidence, the strengths and limitations of individual studies were evaluated based on scientific considerations detailed in Table 12-5 of the Process Appendix (Section 12.6.1). More details on the causal framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA 2015). The evidence presented throughout this appendix supports the following causality conclusions:

Outcome Group	Causality Determination
Immunosuppression	Likely to be Causal
Sensitization and Allergic Responses	Suggestive
Autoimmunity and Autoimmune Disease	Inadequate

The Executive Summary, Integrated Synthesis, and all other appendices of this Pb ISA can be found at https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282.

6.1 Introduction, Summary of the 2013 ISA, and Scope of the Current Review

The 2013 Integrated Science Assessment for Lead (hereinafter referred to as the 2013 Pb ISA) issued causality determinations for the effects of Pb exposure on different aspects of the immune system including atopic and inflammatory responses, decreased host resistance, and autoimmunity (U.S. EPA 2013). It is not without precedent for a single chemical to exert both stimulatory and suppressive effects on various immune parameters (IPCS 2012). The evidence underpinning these causality determinations is briefly summarized below.

The body of epidemiologic and toxicological evidence integrated across the 2013 Pb ISA indicates a "likely to be causal" relationship between Pb exposure and increased atopic and inflammatory conditions. This relationship is supported by evidence for associations of blood Pb levels (BLL) with asthma and allergy in children and Pb-associated increases in immunoglobulin E (IgE) in children and laboratory animals. Uncertainties in the epidemiologic evidence related to potential confounding by

socioeconomic status (SES), smoking, or allergen exposure are reduced through consideration of the evidence from experimental animal studies. The biological plausibility for the effects of Pb on IgE is provided by consistent findings in animals with gestational or gestational-lactational Pb exposures, with some evidence at BLL relevant to humans. These findings are supported by strong evidence of Pb-induced increases in T cell-derived helper (Th)2 cytokine production and inflammation in animals (U.S. EPA 2013).

Available toxicological evidence evaluated in the 2013 Pb ISA indicates a "likely to be causal" relationship between Pb exposure and decreased host resistance. This conclusion was based primarily on animal toxicological studies in which relevant Pb exposures decreased responses to antigens (i.e., suppressed the delayed-type hypersensitivity (DTH) response and increased bacterial titers and subsequent mortality in rodents). Further, evidence demonstrating biological plausibility, including suppressed production of Th1 cytokines and decreased macrophage function in animals support these conclusions (U.S. EPA 2013).

The 2013 Pb ISA also included an evaluation of the epidemiologic and toxicological evidence for Pb-induced autoimmunity. Only a few toxicological studies provided evidence for Pb-induced generation of autoantibodies and the formation of neoantigens that could result in the development of autoantibodies following Pb exposure. Considering the limited evidence at hand, the available studies were inadequate to determine if there is a causal relationship between Pb exposure and autoimmunity (U.S. EPA 2013).

This ISA determined causality for adverse effects of Pb exposure on the three different aspects of the immune system. Accounting for recent toxicological and epidemiologic studies demonstrating that Pb exposure decreases host resistance to infection, suppresses the DTH response in animals, and decreases the vaccine antibody response in children, there is sufficient evidence to conclude that a causal relationship is likely to exist between Pb exposure and immunosuppression. Recognizing that recent epidemiologic studies provide little evidence of an association between exposure to Pb and atopic disease and consistent toxicological evidence that exposure to Pb alters physiological responses in animals consistent with allergic sensitization, the body of evidence supports changing the causal determination from likely to be causal to suggestive of a causal relationship between Pb exposure and sensitization and allergic responses. Evidence for effects of Pb exposure on autoimmunity and autoimmune disease are disparate and highly limited. For that reason, the body of evidence describing the relationship between exposure to Pb and autoimmunity remains inadequate to determine if a causal relationship exists.

The following sections provide an overview of study inclusion criteria for this appendix (Section 6.2), summaries of recent health effects evidence (Sections 6.3, 6.4, and 6.5), a discussion of biological plausibility (Section 6.6), and a discussion of the causality determination for Pb exposure and immune system effects (Section 6.7, Table 6-1, Table 6-2, and Table 6-3).

6.2 Scope

The scope of this appendix is defined by Population, Exposure, Comparison, Outcome, and Study Design (PECOS) statements. The PECOS statement defines the objectives of the review and establishes study inclusion criteria, thereby facilitating identification of the most relevant literature to inform the Pb ISA.[1] In order to identify the most relevant literature, the body of evidence from the 2013 Pb ISA was considered in the development of the PECOS statements for this appendix. Specifically, well-established areas of research; gaps in the literature; and inherent uncertainties in specific populations, exposure metrics, comparison groups, and study designs identified in the 2013 Pb ISA inform the scope of this appendix. The 2013 Pb ISA used different inclusion criteria than the current ISA, and the studies referenced therein often do not meet the current PECOS criteria (e.g., due to higher or unreported biomarker levels). Studies that were included in the 2013 Pb ISA, including many that do not meet the current PECOS criteria, are discussed in this appendix to establish the state of the evidence prior to this assessment. With the exception of supporting evidence used to demonstrate the biological plausibility of Pb-associated effects on the immune system, recent studies were only included if they satisfied all of the components of the following discipline-specific PECOS statements:

Epidemiologic Studies:

Population: Any human population, including specific populations or lifestages that might be at increased risk of a health effect;
Exposure: Exposure to Pb[2] as indicated by biological measurements of Pb in the body – with a specific focus on Pb in blood, bone, and teeth; validated environmental indicators of Pb exposure[3]; or intervention groups in randomized trials and quasi-experimental studies;
Comparison: Populations, population subgroups, or individuals with relatively higher versus lower levels of the exposure metric (e.g., per unit or log unit increase in the exposure metric, or categorical comparisons between different exposure metric quantiles);
Outcome: Immune system effects including but not limited to immunotoxicity, systemic inflammation, and immune-based diseases; and
Study Design: Epidemiologic studies consisting of longitudinal and retrospective cohort studies, case-control studies, cross-sectional studies with appropriate timing of exposure for the health

[1] The following types of publications are generally considered to fall outside the scope and are not included in the ISA: review articles (which typically present summaries or interpretations of existing studies rather than bringing forward new information in the form of original research or new analyses), Pb poisoning studies or clinical reports (e.g., involving accidental exposures to very high amounts of Pb described in clinical reports that may be extremely unlikely to be experienced under ambient air exposure conditions), and risk or benefits analyses (e.g., that apply concentration-response functions or effect estimates to exposure estimates for differing cases).
[2] Recent studies of occupational exposure to Pb were considered insofar as they addressed a topic area that was relevant to the National Ambient Air Quality Standards review (e.g., longitudinal studies designed to examine recent versus historical Pb exposure).
[3] Studies that estimate Pb exposure by measuring Pb concentrations in particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 μm^3 (PM_{10}) and particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm^3 ($PM_{2.5}$) ambient air samples are only considered for inclusion if they also include a relevant biomarker of exposure. Given that size distribution data for Pb-PM are fairly limited, it is difficult to assess the representativeness of these concentrations to population exposure [Section 2.5.3 (U.S. EPA 2013)]. Moreover, data illustrating the relationships of Pb-PM_{10} and Pb-$PM_{2.5}$ with BLLs are lacking.

endpoint of interest, randomized trials, and quasi-experimental studies examining interventions to reduce exposures.

Experimental Studies:

Population: Laboratory nonhuman mammalian animal species (e.g., mouse, rat, guinea pig, minipig, rabbit, cat, dog) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages);
Exposure: Oral, inhalation, or intravenous treatment(s) administered to a whole animal (in vivo) that results in a BLL of 30 µg/dL or below;[1,2]
Comparators: A concurrent control group exposed to vehicle-only treatment or untreated control;
Outcome: Immunological effects; and
Study Design: Controlled exposure studies of animals in vivo.

Consistent with this scoping, the following sections evaluate evidence for the effects of Pb exposure on the immune system. In the 2013 Pb ISA, evidence for effects on the immune system was organized into atopic and inflammatory responses, decreased host resistance, and autoimmunity. Immunological evidence for this ISA is organized to reflect disease categories most relevant to Pb exposure including immunosuppression (Section 6.3), sensitization and allergic responses (Section 6.4), and autoimmunity and autoimmune diseases (Section 6.5). These categories encapsulate the immune-related endpoints used in the 2013 Pb ISA while recognizing advances in the field of immunotoxicology.

The sections that follow focus on studies published since the completion of the 2013 Pb ISA. This evidence is organized and weighed based on the World Health Organization's *Guidance for Immunotoxicity Risk Assessment for Chemicals* (IPCS 2012). As detailed in this guidance, data from endpoints observed in the absence of an immune stimulus (e.g., levels of serum immunoglobulins, white blood cell (WBC) counts, WBC differentials, T cell subpopulations, immune organ weights) are not sufficient on their own to draw a conclusion regarding immune hazard but may provide useful supporting evidence, especially when evaluated in the broader context of functional data (IPCS 2012). Consequently, the sections that follow are organized into two categories: the more informative measures of immune system function and supporting immune system data. Study-specific details, including animal type, exposure concentrations, and exposure durations in experimental studies, and study design, exposure metrics, and select results in epidemiologic studies are presented in evidence inventories in Section 6.8.

[1] Pb mixture studies are included if they employ an experimental arm that involves exposure to Pb alone.
[2] This level represents an order of magnitude above the upper end of the distribution of U.S. young children's BLLs. The 95th percentile of the 2011–2016 National Health and Nutrition Examination Survey distribution of BLL in children (1–5 years; n = 2,321) is 2.66 µg/dL (CDC 2019) and the proportion of individuals with BLLs that exceed this concentration varies depending on factors including (but not limited to) housing age, geographic region, and a child's age, sex, and nutritional status.

6.3 Immunosuppression

Immunosuppression can lead to the increased incidence and/or severity of infectious and neoplastic diseases. Immunosuppressants may be identified using data generated from general toxicity studies or through completion of dedicated immunotoxicity studies. In either case, evidence may be collected from assays designed to assess the function of the immune system following xenobiotic exposure or from observational endpoints that provide supporting information.

6.3.1 Epidemiologic Studies of Immunosuppression

Epidemiologic studies relevant to immunosuppression generally include studies of viral and bacterial infection and vaccine antibody response, as well as studies of WBCs and cytokines. A limited number of epidemiologic studies evaluated in the 2013 Pb ISA (U.S. EPA 2013) provided evidence of associations between cord blood or blood Pb and viral and bacterial infection in children. However, these studies were cross-sectional and did not include adjustment for potential confounders, limiting the strength of conclusions that could be drawn about the effects of Pb exposure on viral or bacterial infections. Cross-sectional studies of cell-mediated immunity reported consistent associations between BLL and lower T cell abundance in children, while results from other studies on lymphocyte activation, macrophages, neutrophils, and natural killer (NK) cells were generally inconsistent or not sufficiently informative (e.g., cross-sectional study designs with limited or no consideration of potential confounding and a lack of information on concentration-response relationships).

There have been a number of recent epidemiologic studies of immunosuppression, including prospective birth cohorts and studies with lower mean or median BLL than those reviewed in the 2013 Pb ISA, many with measures of central tendency <2 μg/dL. The recent studies also apply more robust statistical methods and consistently consider a wider range of potential confounders. In general, recent studies provide consistent evidence that exposure to Pb is associated with increased susceptibility to infection and reduced vaccine antibody response. Additionally, a group of studies in the same population provides some evidence of altered immune cells and cytokines in association with BLL. Measures of central tendency for BLL used in each study, along with other study-specific details, including study population characteristics and select effect estimates, are highlighted in Table 6-4. An overview of the recent evidence is provided below.

6.3.1.1 Host Resistance

While the 2013 Pb ISA (U.S. EPA 2013) evaluated a limited number of epidemiologic studies that indicated an association between BLL and viral and bacterial infections in children, none of the studies considered potential confounders and most analyzed populations with higher BLL (means

>10 µg/dL). Recent studies expand the evidence base by examining populations with wider age-ranges and much lower mean and median BLL. The recent studies also adjust for a wide range of potential confounders, including extensive consideration of SES factors.

Recent cross-sectional studies provide consistent evidence of associations between Pb exposure and viral and bacterial infections, including *Helicobacter Pylori*, *Toxoplasma Gondii*, and Hepatitis B (Park et al. 2020; Krueger and Wade 2016), or susceptibility to antibiotic resistance measured via nasal *Staphylococcus aureus* colonization (Eggers et al. 2018). In a National Health and Nutrition Examination Survey (NHANES) analysis including children and adults, a 1 µg/dL increase in BLL was associated with 8 to 10% increased odds of *H. Pylori* (odds ratio [OR]: 1.09 [95% confidence interval (CI): 1.05, 1.13]), *T. Gondii* (OR: 1.10 [95% CI: 1.06, 1.14]), and Hepatitis B (OR: 1.08 [95% CI: 1.03, 1.13]) seropositivity in the U.S. population (Krueger and Wade 2016). Positive associations were persistent, but varied in magnitude across more specific age groups, including children under 13, participants aged 13 to 35, and adults ≥35 years old. The associations for *H. Pylori* were markedly stronger in magnitude for children less than 13 years old compared with the other age groups, whereas the associations for *T. Gondii* were slightly weaker in children. Additionally, in multipollutant models with cadmium (Cd), there was no evidence to suggest additive or multiplicative interaction between Pb and Cd. Another cross-sectional study of adults with abnormal lesions identified during endoscopy also reported that *H. Pylori* infection rates were associated with increased BLL (Park et al. 2020).

In addition to cross-sectional studies, a recent test-negative case-control study reported that peak BLLs were associated with increases in influenza and respiratory syncytial virus (RSV) rates in children <4 years old presenting with relevant symptomology (Feiler et al. 2020). Test-negative case-control study designs are often used in vaccine efficacy studies to control for healthcare seeking behaviors, but for the intended purposes of this study, the design could bias results toward the null if the non-RSV and influenza illnesses are also related to Pb-induced immune deficiencies. The results in the full population were adjusted for fewer potential confounders (i.e., age, sex, race, ethnicity, insurance status, and season) on account of missing variables, and the observed associations were null in a notably reduced sample population (<25%) with expanded adjustment for confounders.

6.3.1.2 Antibody Responses

There were no studies evaluated in the 2013 Pb ISA (U.S. EPA 2013) that examined the relationship between exposure to Pb and vaccine antibody response in children. There are a few recent studies that provide generally consistent evidence of Pb-related decreases in vaccine antibodies in populations with low mean or median BLL.

In a birth cohort of vaccinated children in South Africa, Di Lenardo et al. (2020) reported that a 1 µg/dL increase in BLL at age 1 was associated with a 13% (95% CI: 2%, 26%) increase in the risk of tetanus IgG titers below the protective limit at age 3.5 years. A key strength of this study was its

prospective nature and the timing of blood Pb measures that approximately coincided with vaccine administration. The authors also examined measles and *Haemophilus influenzae* type B (Hib) IgG levels but did not observe associations with BLL. Cross-sectional studies— including a large NHANES analysis of children ages 6 to 17 years old (Jusko et al. 2019) and another small study comparing kindergarten-aged children in China living near an e-waste facility to those in a nearby community with similar sociodemographic characteristics (Xu et al. 2015)— also provide evidence of Pb-associated decreases in virus-neutralizing antibodies. However, unlike the results from Di Lenardo et al. (2020), Jusko et al. (2019) reported that increased BLLs were associated with decreases in anti-measles IgG antibodies, as well as anti-mumps antibodies. The authors observed a null association with anti-rubella IgG levels. In the analysis in China, Xu et al. (2015) noted that geometric mean BLL dropped precipitously between the 2 years of the study (>3 μg/dL). The authors conducted an analysis stratified by the year of the study and observed decreased antibody to Hepatitis B surface antigen (HBsAb) titers in relation to increases in BLL in both years; however, the association was notably stronger in magnitude in the year with higher geometric mean BLL (2011: −0.447 s/co [95% CI: −0.491, −0.403 s/co]; 2012: −0.366 s/co [95% CI: −0.404, −0.328 s/co] per 1 μg/dL increase in blood Pb). A notable uncertainty in this analysis is potential confounding by other contaminants present in the community. In contrast to the previously discussed evidence, a birth cohort of vaccinated children in Bangladesh reported a positive association between cord BLL and diphtheria and tetanus IgG antibodies at age 5 (Welch et al. 2020). Notably, the associations were null when the exposure metric was concurrent blood Pb rather than cord blood Pb.

6.3.1.3 White Blood Cells and Cytokines

Several epidemiologic studies evaluated in the 2013 Pb ISA (U.S. EPA 2013) examined the relationship between Pb exposure and changes in WBC populations (i.e., counts and phenotypes) and cytokine levels. Although WBC counts and cytokine levels are commonly evaluated in epidemiologic studies, these data can be challenging to interpret because (1) WBC populations are not particularly sensitive indicators of immunotoxicity and (2) changes in cytokine levels can be associated with many different types of tissues and toxicities, either as part of cell differentiation to different immune cell types or including site-specific inflammation, which reflects an immune response to tissue injury but not necessarily an effect on or impairment of immune function (Tarrant 2010). For these reasons, WBC populations and cytokine secretion data (in the absence of a stimulus) are not considered apical outcomes for the purpose of identifying immune hazard, but rather as supporting evidence for understanding mechanisms of immune disruption.

There was generally consistent evidence of associations between increased BLLs and T cell counts in children, but epidemiologic evidence for other immune cell and cytokine measures were uninformative due to cross-sectional study designs with limited or no consideration of potential confounding and a lack of information on the concentration-response relationship. Recent studies provide

some evidence of Pb-related changes in immune cell and cytokine abundance in children, though the number of studies examining overlapping immunological markers is limited.

The majority of recent epidemiologic studies of WBCs and cytokines come from a group of related, small cross-sectional studies evaluating a study population of kindergarten-aged children in Guangdong, China living either near an e-waste facility or in a nearby community with otherwise similar sociodemographic characteristics and pollutant exposures (Chen et al. 2021; Zhang et al. 2020; Huo et al. 2019; Cao et al. 2018; Dai et al. 2017). Across these studies, authors reported that increases in BLL were associated with changes in a number of biomarkers related to immunological function, including increases in the proinflammatory cytokines interleukin (IL)-1β (Zhang et al. 2020; Huo et al. 2019), IL-12p70, and interferon (IFN)-γ (Huo et al. 2019) and pleiotropic cytokine IL-6 (Zhang et al. 2020). Chronic inflammation has the potential to contribute to the development of immunosuppression (Kanterman et al. 2012). In addition, increases in BLL were associated with changes in other biomarkers of immune system function including increases in erythrocyte complement receptor type 1 (CR1) expression (Dai et al. 2017); percentage of cluster of differentiation (CD)4+ central memory T cells (Cao et al. 2018); neutrophils (Zhang et al. 2020); and WBCs, neutrophils, and monocytes (Chen et al. 2021); and decreases in the percentage of $CD4^+$ naive T cells (Cao et al. 2018) and tumor necrosis factor alpha (TNF)-α (Zhang et al. 2020). The authors of these studies also noted some null associations with BLL, including $CD3^+$, $CD4^+$, and $CD8^+$ cell counts (Cao et al. 2018) and monocytes, lymphocytes, IL-8, and IL-10 (Zhang et al. 2020). Consistent with Chen et al. (2021), another cross-sectional study in China with a similar design (e.g., kindergartners recruited from reference and control communities with and without industrial exposure to Pb) reported null associations between BLL and odds of decreased WBC counts (Li et al. 2018).

In the only recent study of an adult population, a small cross-sectional analysis of oil spill response workers with low BLL (mean: 1.82 μg/dL), Werder et al. (2020) observed Pb-associated increases proinflammatory cytokines (i.e., IL-1β and IL-8) and pleiotropic cytokine IL-6 but not the proinflammatory cytokine TNF-α. This was generally consistent with the previously discussed results in children, with the exception of IL-8 for which a null association was reported in children. Notably, as highlighted in a stratified analysis, the observed associations are entirely driven by associations in obese participants Werder et al. (2020). For example, a 1 μg/dL increase in BLL was associated with a 72.8 pg/mL (95% CI: 36.9, 108.7 pg/mL) increase in IL-6 in the entire study population. However, in the stratified analysis, the association was stronger in magnitude in obese participants (169.6 pg/mL [95% CI: 119.8, 219.4 pg/mL]) and null in non-obese participants (−2.6 pg/mL [95% CI: −45.5, 40.3 pg/mL]).

6.3.2 Toxicological Studies of Immunosuppression

Toxicological studies evaluated in the 2013 Pb ISA (U.S. EPA 2013) investigating Pb-induced immunosuppression were derived from several lines of evidence including functional assays (i.e., host

resistance, antibody responses, DTH response, and ex vivo WBC function) and supported by various forms of observational data. Some of these data were reviewed in the 2006 Air Quality Criteria for Lead (AQCD) (U.S. EPA 2006). Based on these previous evaluations, there is clear evidence that exposure to Pb decreases host resistance to bacterial infection and increases production of some pathogen-specific antibody subtypes promoting the shift toward Th2-type immune responses. The results of investigations of the T cell dependent antibody response were inconsistent, with one study reporting a decrease in the antibody response (BLL not reported) and another showing no effect in mice with high BLLs (i.e., 59–132 μg/dL). However, Pb has consistently been shown to suppress the DTH response in animal models. Pb exposure also affected the functions of various WBCs under ex vivo conditions leading to (1) suppression of Th1-mediated immunity (i.e., suppressed Th1 cytokine production (e.g., IFN-γ) and DTH response); (2) altered macrophage function (e.g., increased reactive oxygen species [ROS] production, decreased nitric oxygen [NO] production); and (3) reduced monocyte/macrophage phagocytosis. In addition to assessing the effect of Pb on measures of immune system function, the effects of Pb exposure on various immunotoxicology-related observational endpoints were also evaluated, including (1) total serum immunoglobulins, (2) immune organ weight, (3) WBC number in the spleen, thymus, lymph nodes, and bone marrow, and (4) WBC counts and subpopulation data collected from blood samples. Generally, the number of these studies was limited and differences in study design and the specific endpoints measured create challenges when interpreting these observational data.

Recent toxicological studies are limited in number and report on disparate outcomes, but generally support evidence reported in the previous Pb ISA. Consistent with findings reported in the previous ISA, Pb exposure was again shown to suppress the DTH response. There are no recent toxicology studies investigating the effects of Pb exposure on host resistance; however, there is some recent evidence that Pb exposure altered the levels of some classes of antigen-specific antibodies in iron-deficient rats. Pb exposure also reduced the total serum levels of some immunoglobulins in rats. As with the previous ISA, the effects of Pb on immune organ pathology and spleen weight were inconsistent. New to this ISA, a recent study reported that Pb exposure decreased relative thymus weight. Differences in experimental design and the specific types of WBCs assessed complicate interpretation of data collected on the number and relative abundance of the different types of WBCs in the spleen, thymus, lymph nodes, and bone marrow following exposure to Pb. WBC counts and subpopulation data collected from hematological investigations are similarly challenging to interpret.

6.3.2.1 Host Resistance

Available toxicological evidence evaluated in the 2013 review provides clear evidence that host resistance to bacterial infection is compromised following Pb exposures, resulting in BLLs as low as 20 μg/dL. The 2013 Pb ISA (U.S. EPA 2013) reported several rodent host resistance studies wherein mortality was increased in pathogen-exposed animals that were also exposed to Pb through drinking water. For example, various studies reported decreased clearance of bacteria and increased mortality

induced by *Listeria monocytogenes* in mice exposed postnatally to Pb acetate in drinking water for 3 to 8 weeks, resulting in BLLs ranging from 20–25 µg/dL (Dyatlov and Lawrence 2002; Kim and Lawrence 2000; Kishikawa et al. 1997; Lawrence 1981). Other studies reported increased mortality from *Salmonella* or *Escherichia. Coli*, or decreased clearance of Staphylococcus, in mice administered Pb acetate or Pb nitrate via injection, resulting in BLLs relevant to the 2013 Pb ISA (Fernandez-Cabezudo et al. 2007; Bishayi and Sengupta 2006; Cook et al. 1975; Hemphill et al. 1971; Selye et al. 1966). In addition to high BLL (i.e., 71–313 µg/dL), increased mortality from viral infection was also reported in mice and chickens administered Pb (mostly Pb acetate) for 4–10 weeks (Gupta et al. 2002; Exon et al. 1979; Thind and Khan 1978). Further, evidence suggested a plausible mode of action involving suppressed production of Th1 cytokines (Fernandez-Cabezudo et al. 2007; Lara-Tejero and Pamer 2004), decreased macrophage function (Lodi et al. 2011; Bishayi and Sengupta 2006; Chen et al. 1997; Hilbertz et al. 1986; Castranova et al. 1980), and increased inflammation in animals (Miller et al. 1998; Baykov et al. 1996; Zelikoff et al. 1993).

There were no recent toxicology studies investigating the effects of Pb exposure on host resistance that satisfied the PECOS criteria described in Section 6.2 available for this review.

6.3.2.2 Delayed-Type Hypersensitivity Responses

Antigen-specific cell-mediated immune (CMI) responses are a key component of host defense mechanisms against virally infected cells, tumor cells, and certain fungal infections. The DTH assay is a standard test for assessing CMI responses in animals (IPCS 2012). As noted in the 2013 Pb ISA, suppressed DTH response is one of the most consistently reported immune effects associated with Pb exposure in animals (U.S. EPA 2013). Suppression of the DTH response has been reported following gestational (Chen et al. 2004; Bunn et al. 2001a; Bunn et al. 2001b; Bunn et al. 2001c; Lee et al. 2001; Chen et al. 1999; Miller et al. 1998; Faith et al. 1979) and postnatal (McCabe et al. 1999; Laschi-Loquerie et al. 1984; Müller et al. 1977) exposures to Pb acetate resulting in BLLs ranging from 6.75 to >100 µg/dL) in rats, mice and chickens (U.S. EPA 2013).

In a recent study, administration of Pb acetate in drinking water for 42 days (BLL = 18.48 µg/dL) significantly suppressed the DTH response in adult male Sprague Dawley rats (Fang et al. 2012). To explore the role of regulatory T cells (Tregs) in the DTH response, Fang et al. (2012) employed a T cell transfer model. Total CD4+ T cells and CD4+CD25- cells were collected from control and Pb-exposed rats and then transferred to recipient rats that were subsequently challenged with 1-Fluoro-2,4-dinitrobenzene (DNFB) to induce a DTH response. The DTH response was diminished in rats receiving CD4+ T cells from Pb-exposed rats compared with those receiving CD4+ cells from control animals. Importantly, the effect was lost when Tregs were depleted from the pool of CD4+ cells transferred to the recipient rats. These findings suggest that Tregs play a critical role in Pb-induced immune suppression

(Fang et al. 2012). Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-6.

6.3.2.3 Antibody Responses

The production of antigen-specific antibodies is a major defense mechanism of humoral immune responses. Only one study reporting effects on antigen-specific antibody responses was evaluated in the 2013 Pb ISA (U.S. EPA 2013). In that study, Fernandez-Cabezudo et al. (2007) reported no difference in the serum levels of *Salmonella*-specific IgM following infection with a sublethal dose of *Salmonella* (1.5×10^4 organisms/mouse) in control C3H/HeN mice and mice exposed to 10 mM Pb acetate in drinking water for 16 weeks (resultant mean BLL: 106 µg/dL). However, compared with control mice, mice exposed to Pb acetate had less IgG2a and more IgG1 antibodies providing evidence for a shift toward Th2-type immune responses resulting in decreased resistance to *Salmonella enterica* (Fernandez-Cabezudo et al. 2007). Studies describing effects of Pb exposure on the T cell dependent antibody response (TDAR) were also reviewed in the previous ISA. The TDAR is a comprehensive immune function assay that integrates several aspects of immune responses. Thus, xenobiotic-induced alterations in antigen processing and presentation, B and T cell interactions, antibody production, and isotype class switching and modification have the potential to modify this defense mechanism (IPCS 2012). Results of the TDAR response to sheep RBCs have been inconsistent. For example, the TDAR was significantly decreased in mice exposed to Pb acetate through drinking water for 3weeks, resulting in BLLs of 25.4 µg/dL (Blakley and Archer 1981). However, in a second drinking water study, the TDAR was increased in 1 of 8 mouse strains (the other 7 strains were unaffected) evaluated following administration of Pb acetate in drinking water for 8 weeks resulting in high BLL (mean range 59–132 µg/dL) (Mudzinski et al. 1986).

In a recent study, adult Sprague Dawley rats (data from both sexes pooled) were fed either a control diet or an iron-deficient diet for the duration of the experiment (Yathapu et al. 2020). After confirming iron deficiency at 4 weeks, rats were administered Pb acetate in drinking water for 4 weeks. At this time, a subset of mice was vaccinated with tetanus toxoid (TT). Rats received two booster doses (2-week interval) before assessing antigen-specific antibody levels 2 weeks after the last booster dose. Under these conditions, Pb acetate (BLL = 16.1 µg/dL) had no effect on the levels of anti-TT-specific IgG and IgM antibodies in the serum of rats that received the control diet whereas the levels of anti-TT-specific IgM were decreased and those of IgG were unaffected in the serum of iron-deficient rats (Yathapu et al. 2020). Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-5.

6.3.2.4 Ex Vivo White Blood Cell Function

White blood cells are cells of the immune system involved in protecting the body from infectious disease. These cells can be organized into two lineages— myeloid cells and lymphoid cells. Myeloid cells (i.e., myelocytes) include neutrophils, eosinophils, mast cells, basophils, and monocytes. Lymphoid cells (i.e., lymphocytes) include T cells, B cells, and NK cells. Xenobiotic-induced alterations in ex vivo WBC function is considered clear evidence of immunosuppression (IPCS 2012). Ex vivo WBC function assays are performed outside the body using immune cells collected from exposed individuals.

The 2013 Pb ISA reviewed the effects of Pb exposure on the functions of various WBCs under ex vivo conditions indicating (1) a shift in lymphocyte cytokine production towards the production of Th2 cytokines (Heo et al. 2007; McCabe and Lawrence 1991), reduced number of Th1 cells and Th1 cytokine levels (McCabe and Lawrence 1991), (2) increased dendritic cell induced Th2 cell proliferation and cytokine production (Gao et al. 2007), and (3) reduced monocyte/macrophage phagocytosis (Lodi et al. 2011; Bussolaro et al. 2008; Deng and Poretz 2001; Kowolenko et al. 1991; Zhou et al. 1985) and decreased NO production (Farrer et al. 2008; Mishra et al. 2006; Bunn et al. 2001b; Lee et al. 2001; Krocova et al. 2000; Chen et al. 1997; Tian and Lawrence 1996; Tian and Lawrence 1995). No studies on neutrophils and NK cells were reviewed in the 2013 Pb ISA.

A few PECOS-relevant papers evaluating the effects of Pb exposure on ex vivo WBC function have been published since the 2013 ISA. Fang et al. (2012) reported that administration of Pb acetate in drinking water for 42 days (BLL = 18.48 µg/dL) had no effect on the suppressive properties of Tregs isolated from adult male Sprague Dawley rats. In a second study, the effects of Pb administration on Concanavalin A (Con A)-stimulated lymphocyte proliferation and cytokine production were investigated (Yathapu et al. 2020). For this investigation, adult male and female Sprague Dawley rats were fed either a control diet or an iron-deficient diet for the duration of the experiment. After confirming iron deficiency at 4 weeks, the rats were administered Pb acetate in drinking water for 4 weeks. At this time, a subset of rats was vaccinated with TT. Rats received two booster doses (2-week interval) before splenocytes were collected 2 weeks after the last booster dose. Irrespective of vaccine status, Pb treatment (BLL = 16.1 µg/dL) had no effect on Con A-stimulated proliferation of splenocytes collected from rats fed the control diet. However, when rats were fed an iron-deficient diet, Pb treatment (BLL = 41.6 µg/dL) increased Con A-stimulated splenocyte proliferation (Yathapu et al. 2020). Unfortunately, because of incomplete reporting, data related to cytokine production by Con A-stimulated splenocytes reported by Yathapu et al. (2020) are not interpretable. In addition, Cai et al. (2018) measured cytokine levels directly in blood and reported that, administration of Pb acetate drinking water (0.2%; BLL = 9.3 µg/dL) for 84 days had no effect on erythropoietin, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6, and TNF-α levels in adult Sprague Dawley rats (data from sexes pooled). Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-7 and Table 6-14.

6.3.2.5 Immune Organ Pathology

The 2013 Pb ISA did not report on the effects of Pb exposure on immune organ pathology (U.S. EPA 2013). However, xenobiotic exposure can alter primary immune sites important for immune cell maturation, including the bone marrow, liver, thymus, and Peyer's patches. Secondary lymphoid sites (i.e., spleen, lymph nodes, tonsils) can also be affected by exposure to immunotoxicants. Data from these endpoints are not sufficient on their own to draw a conclusion regarding immune hazard, but may provide useful supporting evidence (IPCS 2012). Pb-induced alterations in immune organ pathology were not addressed in the 2013 Pb ISA.

Since the 2013 Pb ISA, there have been three reports published that included an assessment of immune organ pathology following exposure to Pb and that fit the PECOS criteria described in Section 6.2. In the first study, Pb treatment induced changes in the spleen architecture of adult male C57BJ mice exposed via drinking water (200 ppm; BLL = 21.6 µg/dL) for 45 days. These changes included increasing the amount of white pulp (qualitative) and decreasing the definition of the germinative center of the inner peri-arteriolar lymphoid sheath, but the marginal zone was unaffected (Corsetti et al. 2017). In a different study, inhalation of Pb oxide nanoparticles ($1.23 \times 10^6 \times 10$ particles/cm^3, 24 hours/day for 6 weeks BLL 13.9 µg/dL) had no effect on spleen pathology in two experiments conducted in adult female Institute for Cancer Research (ICR) mice (Dumková et al. 2017). Dumková et al. (2020a) conducted another study with Pb oxide nanoparticles (68.6×10^6 particles/cm^3, 24 hours/day for up to 6 weeks) in CD-1(ICR) mice that included histological analysis of the spleen, but did not report their findings. Exposure to Pb oxide nanoparticles (0.956×10^6 particles/cm^3, 24 hours/day for 11 weeks, BLL = 18.1 ∝g/dL) had no effect on spleen histopathology in CD-1(ICR) BR mice (Smutná et al. 2022). Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-8.

6.3.2.6 Immunoglobulin Levels

Immunoglobulins (i.e., antibodies) are produced by plasma cells (i.e., differentiated B cells). Immunoglobulins are a critical part of the immune response and act by recognizing and binding to specific antigens such as bacteria and viruses leading to their destruction. Although immunoglobulin type and quantity are easy to measure in serum, their levels are difficult to interpret in the absence of a controlled immune challenge. For this reason, these data are not considered a predictive measure for immunotoxicity and are most useful for supporting data collected from immune functional assays. The 2013 Pb ISA reviewed the effects of Pb exposure on total serum IgE in the context of immediate-type hypersensitivity (Chen et al. 2004; Snyder et al. 2000; Miller et al. 1998; Heo et al. 1997; Heo et al. 1996). In addition, the 2013 ISA reviewed the effects of Pb exposure on total serum IgG subtypes (Kasten-Jolly et al. 2010; Carey et al. 2006; Gao et al. 2006; Snyder et al. 2000). While noting that the

BLLs were not relevant to human exposures, the 2013 Pb ISA described the observed effects as inconsistent.

Since the 2013 Pb ISA, only one PECOS-relevant publication included an assessment of total serum immunoglobulin levels following exposure to Pb. For this investigation, adult Sprague Dawley (data from sexes pooled) were fed either a control diet or an iron-deficient diet for the duration of the experiment. After confirming iron deficiency after 4 weeks, rats were administered Pb acetate in drinking water for 4 weeks. At this time, a subset of mice was vaccinated with TT. Rats received two booster doses (2-week interval) before splenocytes were collected 2 weeks after the last booster dose. Irrespective of vaccine status, Pb treatment reduced mucosal IgA levels in rats fed the control diet (BLL = 16.1 µg/dL). Under conditions of iron deficiency, Pb treatment further reduced mucosal IgA levels (BLL = 41.6 µg/dL). Total serum IgM and IgG were unchanged by Pb under all conditions evaluated (Yathapu et al. 2020). Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-9.

6.3.2.7 Immune Organ Weights

Changes in lymphoid organ weights (thymus, spleen, lymph node, or bone marrow) may indicate immunotoxicity and are useful for supporting data collected on immune function. As reported in the 2013 Pb ISA, exposure to Pb increased relative spleen weight in mice and rats exposed to Pb acetate and Pb ion in drinking water (U.S. EPA 2013). In the only available study, lymph node weight decreased following exposure to Pb acetate (Institóris et al. 2006). There were no studies that evaluated changes in thymus weight reviewed in the 2013 Pb ISA. Several recent studies evaluating the effects of Pb exposure on lymphoid tissues are described below, including one study describing effects on the thymus. Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-10.

6.3.2.7.1 Thymus Weight

The thymus, which is essential for T cell development, is a critically important component of the immune system; changes in thymus weight are a more sensitive indicator of immunotoxicity than changes in spleen weight. Relative thymus weight was significantly decreased in juvenile Sprague Dawley rats (data from sexes pooled) orally administered Pb acetate (1 or 10 mg/kg with BLL of 3.27 µg/dL and 12.5 µg/dL, respectively) for up to 25 days (Graham et al. 2011). A second study performed by the same laboratory using the same experimental design investigated the effects of oral administration of Pb acetate (gavage) on relative thymus weight (Amos-Kroohs et al. 2016). Because of incomplete reporting, however, the effect of Pb on thymus weight could not be discerned and this element of the study was rejected for study quality deficiencies.

6.3.2.7.2 Spleen Weight

The spleen has a prominent role in immune function, as well as serving as a reservoir for monocytes. The effect of Pb administration via oral and inhalation routes in rats and mice has been recently investigated. In juvenile Sprague Dawley rats (data from sexes pooled), relative spleen weight was not affected following oral administration of Pb acetate (gavage, 1 or 10 mg/kg with BLL up to 3.27 and 12.5 µg/dL, respectively) for up to 25 days (Amos-Kroohs et al. 2016; Graham et al. 2011). Absolute spleen weight, however, was decreased significantly following exposure to 10 mg/kg (BLL = 12.5 µg/dL) Pb acetate (Graham et al. 2011). Similarly, spleen weight was unaffected in adult male Wistar rats exposed to Pb acetate in drinking water (357 µg/kg/day or 1607 µg/kg/day with BLL of 1.77 ± 0.7 µg/dL and 8.6 ± 2.9 µg/dL, respectively) for 4 weeks (Wildemann et al. 2015). In the only study investigating the effects of Pb exposure in mice, Pb acetate treatment significantly increased relative spleen weight in adult male C57BJ mice exposed via drinking water (200 ppm, BLL = 21.6 µg/dL) for 45 days (Corsetti et al. 2017).

Effects of Pb exposure through inhalation were inconsistent. Inhalation exposure to Pb oxide nanoparticles (1.23×10^6 nanoparticles/cm^3, BLL 13.9 µg/dL) increased relative spleen weight in adult female ICR mice exposed for 6 weeks, but the finding was not replicated in a duplicate experiment performed as part of the same study (Dumková et al. 2017). In a second study performed by the same lead investigator, inhalation exposure to a higher concentration of Pb oxide nanoparticles (2.23×10^6 nanoparticles/cm^3) for a longer duration (i.e., 11 weeks) had no effect on relative spleen weight adult female CD-1(ICR) BR mice with a BLL of 17.4 µg/dL (Dumková et al. 2020b). However, inhalation exposure to Pb (II) nitrate nanoparticles (68.6×10^6 nanoparticles/cm^3) decreased relative spleen weight in adult female CD-1(ICR) BR mice exposed for 2 weeks (BLL = 4.0 µg/dL), but the effect was not observed at the 6 week or 11-week timepoints with BLL up to 8.5 µg/dL (Dumková et al. 2020a). Similarly, exposure to Pb oxide nanoparticles (0.956×10^6 particles/cm^3, 24 hours/day for 11 weeks, BLL = 18.1 µg/dL) had no effect on relative spleen weight in CD-1(ICR) BR mice (Smutná et al. 2022)

6.3.2.8 White Blood Cell Counts and Differentials (Spleen, Thymus, Lymph node, Bone Marrow)

Changes in WBC number and differentials collected from lymphoid organs may indicate immunotoxicity and are useful for supporting data collected from immune function assays. Although there were no data for WBC counts and differentials in lymphoid tissues reviewed in the 2013 Pb ISA, several recent studies describing the effects of Pb exposure on lymphoid tissues are described below. Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-11.

6.3.2.8.1 Spleen

The effects of Pb exposure on spleen cellularity were investigated in three recent studies. Administration of Pb acetate in drinking water (300 ppm; BLL = 18.48 µg/dL) for 42 days significantly increased the number of Tregs, reduced the absolute number of CD3+ cells and the percentage of CD4+ T cells, but not the percentage CD8+ T cells in the spleens of adult male Sprague Dawley rats (Fang et al. 2012). In contrast, administration of Pb acetate in drinking water for 28 days had no effect on percentage of CD4+ cells, but the percentage of CD8+ cells was significantly increased in the spleens of adult male and female Sprague Dawley rats (BLL = 16.1 µg/dL) (Yathapu et al. 2020). Drinking water exposure to Pb acetate (1250 ppm; BLL 4.7–41.3 µg/dL) for 56 days decreased the number of innate lymphoid cells (ILC), type 1 innate lymphoid cells (ILC1), NK- like ILC1 (NK-ILC1), type 2 innate lymphoid cells (ILC2), and type 3 innate lymphoid cells (ILC3), but Pb had no effect on cell proliferation in vivo in spleens collected from adult male and female (samples pooled) C57BL/6 mice (Zhu et al. 2020).

6.3.2.8.2 Thymus

Pb acetate treatment had no effect on the total number of thymocytes or the number of thymic CD4−/CD8− and CD4+CD8+ cells, but reduced the number of thymic CD4+CD8− cells by 25% and slightly increased the number of CD4−CD8+ cells in adult male Sprague Dawley rats exposed via drinking water (300 ppm; BLL = 18.48 µg/dL) for 42 days (Fang et al. 2012). Administration of Pb in drinking water (300 ppm) for 42 days resulted in a 1.59-fold increase in the number of Tregs in the thymus of adult male Sprague Dawley rats exposed (Fang et al. 2012). There are no other recent studies meeting PECOS criteria available for this endpoint.

6.3.2.8.3 Lymph Node

Two recent studies investigated the effects of Pb exposure on lymph node cellularity. Administration of Pb acetate in drinking water (300 ppm; BLL = 18.48 µg/dL)) to adult male Sprague Dawley rats for 42 days had no effect on the absolute number of CD8+ T cells but reduced the absolute number of CD3+ cells and CD4+ T cells and increased the number of Tregs in the lymph nodes (type not specified) (Fang et al. 2012). Drinking water exposure to Pb acetate (1250 ppm; BLL 4.7–41.3 µg/dL) for 56 days decreased the number of ILCs, ILC1s, NK-like ILC1s (NK-ILC1s), ILC2s, and ILC3s in cervical lymph nodes collected from adult male and female (samples pooled) C57BL/6 mice (Zhu et al. 2020).

6.3.2.8.4 Bone Marrow

Two recent studies investigated the effects of Pb exposure on populations of immune cells in bone marrow. Administration of Pb acetate in drinking water (0.2%; BLL = 9.3 µg/dL) for 84 days had no

effect on the number of CD90+CD45− pluripotent hematopoietic stem cells in bone marrow collected from adult male and female Sprague Dawley rats (Cai et al. 2018). In a second study, administration of Pb acetate in drinking water (1250 ppm; BLL 4.7–41.3 µg/dL) for 56 days decreased the number of ILC progenitors (ILCPs) and reduced number of ILCPs in the bloods of adult C57BL/6 mice (data from sexes pooled) (Zhu et al. 2020). These data suggest that Pb exposure impaired mobilization of ILCP cells to the periphery. In the same study, the number of ILCs, ILC1s, NK-ILC1s, ILC2s, and ILC3s in bone marrow were reduced, but Pb had no effect on cell proliferation in vivo (Zhu et al. 2020). Pb suppressed proliferation of ILCP in bone marrow, however.

To determine if the increase in the number of ILCPs associated with Pb exposure was caused by impeded differentiation, common lymphoid progenitors from the bone marrow of Pb-treated (1250 ppm, 56 days; BLL 4.7–41.3 µg/dL) or control enhanced green fluorescent protein (EGFP) mice were transplanted into Pb-treated or control B6 mice (Zhu et al. 2020). Common lymphoid progenitors collected from Pb-treated EGFP mice gave rise to more ILCs compared with common lymphoid progenitors from control donors in both Pb-treated and control recipients. Furthermore, common lymphoid progenitors from Pb-treated donors produced more mature ILCs in control recipients than in Pb-treated recipients. These findings indicate that common lymphoid progenitors in Pb-treated mice could differentiate into mature ILCs, however, the Pb-treated host environment impeded differentiation into ILCPs.

6.3.2.9 White Blood Cell Counts (Hematology and Subpopulations)

Changes in WBC number and differentials in blood may indicate potential immunotoxicity and are useful for supporting data collected on immune function. The 2013 Pb ISA reviewed one toxicology study that described the effects of Pb exposure on WBC numbers in blood (Sharma et al. 2010). In that study, the total number of WBCs, lymphocytes and monocytes were reduced in male Swiss albino mice treated with Pb nitrate (50 mg/kg/day) (Sharma et al. 2010). The effect of Pb exposure on WBC counts and subpopulations in blood reported in four recent studies are described below. Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-12.

Administration of Pb acetate in drinking water (0.2%, BLL 30.9 ± 14.7 µg/dL) for 1 day had no effect on the number of WBC, lymphocytes and neutrophils in whole blood collected from adult male Wistar rats (Andjelkovic et al. 2019). However, when Pb acetate was administered in drinking water (200 ppm; BLL = 21.6 µg/dL) for 45 days consecutively, the numbers of WBCs, neutrophils, lymphocytes, and eosinophils decreased while the numbers of monocytes and basophils were unchanged in blood collected from adult male C57BJ mice (Corsetti et al. 2017). Changes in WBC number and subpopulations were reported in a second study wherein the total number of WBCs and the number of CD4+ and CD8+ T cells were reduced in blood collected from male and female Sprague Dawley rats (data from sexes pooled) following exposure to Pb acetate in drinking water (0.2%; BLL = 9.3 µg/dL) for

84 days (Cai et al. 2018). Additionally, exposure to Pb acetate (drinking water, 1250 ppm, BLL 4.7–41.3 µg/dL) for 56 days decreased the number of ILCs, type 1 innate lymphoid cells (ILC1), NK-like ILC1 (NK-ILC1), type 2 innate lymphoid cells (ILC2), and type 3 innate lymphoid cells (ILC3). Pb exposure additionally suppressed proliferation of ILCP in blood collected from adult male and female (samples pooled) C57BL/6 mice (Zhu et al. 2020).

6.3.3 Integrated Summary of Immunosuppression

Toxicological evidence for Pb-induced immunosuppression is derived from several lines of evidence including functional assays (i.e., host resistance, antibody responses, DTH response, and ex vivo WBC function) that are supported by various forms of observational data including immunoglobulin levels, immune organ weight, WBC counts and differentials (immune organs), and WBC counts (hematology). Toxicological studies evaluated in the 2013 Pb ISA (U.S. EPA 2013) provide clear evidence that host resistance to bacterial infection is compromised following Pb exposure. Evidence available in 2013 also demonstrated that levels of antigen-specific IgM were unaffected in Pb-exposed mice infected with *Salmonella*. However, levels of IgG2a were decreased and IgG1 antibodies were increased in these mice providing evidence for a shift toward Th2-type immune responses resulting in decreased resistance to *Salmonella*. The potential for Pb exposure to result in immunosuppression was further evaluated using the DTH assay, which has been shown to be consistently suppressed Pb-exposed animals. The effects of Pb administration on the TDAR was also evaluated in the 2013 ISA. Results from these investigations were inconsistent with one study reporting a decrease in the antibody response (BLL not reported) and another showing no effect in mice with high BLLs (i.e., 59–132 µg/dL). The effects of Pb exposure on the functions of various WBCs under *ex vivo* conditions indicated that Pb exposure results in (1) suppression of Th1-mediated immunity (i.e., suppressed Th1 cytokine production [e.g., IFN-γ] and DTH response); (2) altered macrophage function (e.g., increased ROS production, decreased NO production); and (3) reduced monocyte/macrophage phagocytosis.

The 2013 Pb ISA also described toxicological evidence for effects of Pb exposure on various observational endpoints (e.g., total serum immunoglobulins, immune organ weights, WBC counts) that support data derived from immune function assays. Investigations of these endpoints are limited in number, however, and due to differences in experimental design, challenging to interpret. For example, inconsistent effects of Pb exposure on total serum IgE and IgG subtypes were described in the previous ISA. Data reporting effects of Pb exposure on immune organ weight were limited to one study reporting increased relative spleen weight and another study reporting decreased lymph node weight following Pb exposure. Additional studies investigated the number and relative abundance of different types of WBC in the spleen, thymus, lymph nodes and bone marrow following exposure to Pb, although study design limitations and differences in the types of WBC assessed limit our ability to interpret these data. In the only study reporting on WBC counts and subpopulation data collected in blood reviewed in the previous ISA, Pb exposure reduced the total number of WBC, lymphocytes, and monocytes.

The epidemiologic studies relevant to immunosuppression that were evaluated in the 2013 Pb ISA (U.S. EPA 2013) were more limited in number than the available toxicological evidence base. Irrespective, these studies indicated some evidence of an association between BLLs and viral and bacterial infections in children. None of the studies considered potential confounders, however, and most analyzed populations with higher BLLs (means >10 μg/dL). As described in the 2013 ISA, some epidemiologic studies also examined the effects of Pb exposure on WBC populations and cytokine levels. Evaluation of these provided generally consistent evidence of associations between increased BLLs and lower T cell abundance in children, though most associations were seen with higher concurrent BLLs (>10 μg/dL). These results were coherent with the toxicological evidence base. Studies examining macrophages, neutrophils, and NK cells and lymphocyte activation (i.e., HLA-DR expression) were largely uninformative because of limitations associated with consideration of potential confounders and a lack of information on concentration-response relationship.

Since the 2013 ISA, there have been several epidemiologic studies published investigating aspects of immunosuppression. Recent studies investigating associations between Pb exposure and decreased host resistance examine populations with wider age-ranges and much lower mean and median BLLs than studies evaluated in the previous ISA. Recent studies also adjust for a wide range of potential confounders, including extensive consideration of SES factors. Cross-sectional and case-control studies provide consistent evidence of associations between Pb exposure and viral and bacterial infections or susceptibility to antibiotic resistance. Antibody response, an endpoint that was not examined in studies evaluated in the previous ISA, was investigated in several recent studies. Specifically, a birth cohort study and a few cross-sectional studies demonstrate generally consistent evidence of an association between BLLs and decreased virus-neutralizing antibodies. A group of epidemiologic studies examining children in China living either near an e-waste facility or in a nearby community with otherwise similar sociodemographic characteristics and pollutant exposures provides evidence that BLLs are associated with changes in (1) the percentage of $CD4^+$ naive and $CD4^+$ central memory T cells, (2) proinflammatory cytokine levels (IFN-γ, IL-1β, IL-8, IL-10, IL-12p70, and TNF-α), (3) levels of the pleiotropic cytokine IL-6, (4) levels of the anti-inflammatory cytokine IL-10, and (5) the number of neutrophils and monocytes. A few of the studies also reported null associations between BLLs and $CD3^+$, $CD4^+$ and $CD8^+$ cell counts, monocytes, and lymphocytes. The only recent study of an adult population reported similar increases in cytokine levels associated with BLLs.

Available recent studies of immune function generally support evidence reported in the previous Pb ISA. There are no recent toxicology studies investigating the effects of Pb exposure on host resistance available for this review. Exposure to Pb had no effect on levels of anti-TT-specific IgM and IgG antibodies in rats. However, levels of anti-TT-specific IgM (but not IgG) were decreased in iron-deficient rats. Consistent with findings reported in the previous ISA, Pb exposure is again shown to suppress the DTH response. Assessment of the effects of Pb exposure on ex vivo WBC function is limited to assessments of Con A-stimulated lymphocyte proliferation and direct measurement of cytokines in blood. Pb treatment had no effect on Con A-stimulated proliferation of splenocytes collected from rats, however,

treatment increased Con A-stimulated splenocyte proliferation in iron-deficient rats. Pb exposure had no effect on levels of erythropoietin, GM-CSF, IL-6, and TNF-α in a single study performed in rats. Recent studies reporting on the effects of Pb exposure on immune organ pathology were inconsistent, with one study reporting effects on spleen architecture and another showing no effect. Pb exposure reduced total serum IgA immunoglobulins in rats fed a control diet and in iron-deficient rats but had no effect on total serum IgM and IgG in rats fed either diet. Recent investigations also include assessments of the effects of Pb exposure on immune organ weight. Relative thymus weight, which was not evaluated in the previous ISA, decreased following exposure to Pb. As with the previous ISA, the effects of Pb exposure on relative spleen weight were inconsistent, varying with dose, exposure duration, and route of administration (oral versus inhalation). Similarly, because of differences in experimental design and the specific types of WBCs assessed in each study, it is difficult to interpret data collected on the number and relative abundance of the different types of WBCs in the spleen, thymus, lymph nodes and bone marrow following exposure to Pb. WBC counts and subpopulation data collected from hematology investigations are similarly challenging to interpret.

6.4 Sensitization and Allergic Responses

Hypersensitivity responses are the result of an over-reaction of the immune system. Hypersensitivity reactions are organized into four different classes, types I, II, III, and IV (Murphy and Weaver 2016). Irrespective of the type of response, all hypersensitivity responses develop in the same two phases: sensitization and elicitation (or challenge). During the sensitization phase, the immune system is trained to respond to an otherwise innocuous antigen. This phase typically occurs without symptoms. During the elicitation phase, the previously sensitized individual is re-exposed to the antigen precipitating the symptoms of the allergic disease. Important for risk assessors, the concentration of the sensitizing chemical required to elicit an allergic response is, in some cases, orders of magnitude lower than the concentration required for sensitization. Consequently, preventing allergic sensitization from developing in the first place is of paramount importance because dangerous, potentially life-threatening allergic reactions can occur in response to exposure to a prohibitively-low concentration of the sensitizer.

6.4.1 Epidemiologic Studies of Sensitization and Allergic Responses

Epidemiologic studies of sensitization and allergic response generally cover studies of atopic diseases, including asthma, rhinitis, and eczema, as well as studies examining cells and antibodies that mediate these diseases, such as IgE and eosinophils. A limited number of studies evaluated in the 2013 ISA (U.S. EPA 2013) provide evidence of associations between exposure to Pb and asthma and allergic sensitization. The strongest evidence comes from two prospective analyses, one investigating incident asthma requiring medical care (Joseph et al. 2005) and another examining allergic hypersensitization via

skin prick tests (SPTs) (Jedrychowski et al. 2011). Associations in both studies were reported after adjustment for multiple confounders, including sex; birth weight; parity; maternal age, education, and atopy; income; and prenatal and postnatal smoking exposure. Joseph et al. (2005) observed associations between asthma incidence and BLLs ≥5 μg/dL in white children (risk ratio [RR]: 2.7 [95% CI: 0.9, 8.1] compared with white children with BLL <5 μg/dL). In analyses restricted to black children, those with BLLs ≥10 μg/dL had an elevated risk of incident asthma requiring medical care (RR: 1.3 [95% CI: 0.6, 2.6] compared with children with BLLs <5 μg/dL). The effect estimates for both groups were imprecise due to small numbers of children with asthma in the higher BLL categories (five white children with BLLs ≥5 μg/dL and nine black children with BLLs ≥10 μg/dL). Jedrychowski et al. (2011) also reported wide 95% CIs for a 1 μg/dL increase in prenatal cord blood level associated with risk of positive SPT (rash/inflammatory reaction) to dust mite, dog, or cat allergen (RR: 2.3 [95% CI: 1.1, 4.6]). An additional prospective cohort analysis reported an imprecise association between cord BLLs and prevalent asthma in children (Rabinowitz et al. 1990), but did not adjust for potential confounders and had low participation rates with no information on nonparticipants. These findings were supported by a cross-sectional study of cord blood and blood Pb-associated prevalent asthma (Pugh Smith and Nriagu 2011). In addition to studies examining atopic disease incidence or prevalence, the 2013 Pb ISA (U.S. EPA 2013) also includes supporting evidence from population-based cross-sectional studies in children that reported associations between BLL and elevated serum IgE. Notably, many of these studies had limited adjustment for potential confounders and included populations with mean BLLs >5 μg/dL.

There have been several recent epidemiologic studies of sensitization and allergic response, including prospective birth cohorts and cross-sectional studies with mean or median BLLs <2 μg/dL. In general, these recent studies provide little evidence of an association between exposure to Pb and atopic disease, and inconsistent evidence for immunological biomarkers involved in hypersensitivity and allergic response. Measures of central tendency for BLL used in each study, along with other study-specific details, including study population characteristics and select effect estimates, are highlighted in Table 6-13. An overview of the recent evidence is provided below.

Whereas epidemiologic evidence from the previous ISA supported the presence of an association between BLL and incident and prevalent asthma in children, evidence from a few recent studies at lower BLL is not indicative of an association. Specifically, in a small prospective birth cohort in France, Pesce et al. (2021) reported that neither BLL measured during pregnancy nor cord BLL at birth were associated with incident parental-reported asthma attacks through 5 years of age. Notably, there was a low rate of asthma in the study population, limiting the statistical power to detect an association. However, because asthma can be difficult to diagnose in children under 5, asthma attacks may be the most reliable measure. In a cross-sectional NHANES analysis including slightly older children (2–12 years old), Wells et al. (2014) also observed a null association between BLL and prevalent asthma.

Other recent epidemiologic studies of atopic disease are also generally consistent in reporting a lack of an association with low levels of exposure to Pb. A few birth cohorts (Kim et al. 2019; Kim et al.

2013) and a cross-sectional NHANES analysis including respondents of all ages (Wei et al. 2019) did not observe associations between cord blood or BLL and eczema incidence or prevalence. While Pesce et al. (2021) reported a null association between maternal BLL and eczema in the aforementioned French birth cohort, the authors did note substantial increases in the odds of eczema incidence for children in the higher quartiles of cord blood Pb exposure compared with the lowest quartile. However, given the range of outcomes examined (which included null associations for rhinitis and food allergy, in addition to asthma) and the use of two exposure metrics (maternal blood and cord blood), the eczema results could be an artifact of multiple testing. Consistent with Pesce et al. (2021), Mener et al. (2015) also reported a null association between BLL and food allergies in children. However, the authors noted a 10% increase in odds of food allergy sensitization in adults per 1 μg/dL increase in BLL (95% CI: 1%, 20%). In a restricted cubic spline model, the observed relationship was approximately linear across the range of lower BLL (<3 μg/dL), with no evidence of a threshold.

Results from a limited number of recent epidemiologic studies of allergen-specific and non-specific immunological biomarkers of hypersensitivity in adults are inconsistent. A cross-sectional Korea National Health and Nutrition Examination Survey (KNHANES) analysis reported an increase in total IgE concentrations associated with a 1 μg/dL increase in BLL in adults (Kim et al. 2016). Notably, the observed increases were stronger in magnitude in respondents with house dust mite sensitization (10.4% [95% CI: 3.3%, 17.8%]) compared with those without (3.5% [95% CI: −1.8%, 9.4%]). No other recent studies examined total IgE levels in adults, although Tsuji et al. (2019) reported that BLLs were not associated, or slightly negatively associated, with allergen-specific serum IgE concentrations in pregnant women, including egg white, hose dust mite, Japanese cedar pollen, animal dander, and moth allergens. The interpretation of the results is complicated, however, by timing of the exposure and outcome, where IgE concentrations were measured earlier in pregnancy (first trimester) than BLL (second or third trimester).

Recent epidemiologic studies of non-specific immunological biomarkers of hypersensitivity in neonates and children also provide inconsistent evidence of an association with exposure to Pb. In a small birth cohort in south Korea, Kim et al. (2019) observed a cross-sectional association between increased cord BLL and increased cord blood IL-13. In another cross-sectional analysis, Wells et al. (2014) reported that a 1 μg/dL increase in blood Pb was associated with a 10.3% (95% CI: 3.5%, 17.5%) increase in serum total IgE and a 4.6% (95% CI: 2.4%, 6.8%) increase in percent eosinophils. In contrast, results from a larger birth cohort in Canada did not indicate increased odds of elevated cord blood IgE concentrations in relation to increases in average BLL across the first and third trimesters of pregnancy (Ashley-Martin et al. 2015). Further, the authors reported an inverse association between pregnancy BLL and odds of simultaneously elevated cord blood IL-33 and thymic stromal lymphopoietin (TSLP).

6.4.2 Toxicological Studies of Sensitization and Allergic Responses

The 2013 Pb ISA reviewed evidence for the ability of Pb to induce immediate-type hypersensitivity leading to the development of allergic asthma (U.S. EPA 2013). Available studies reported that exposure to Pb increased lymph node cell proliferation, increased production of Th2 cytokines such as IL-4, increased total serum IgE antibody levels in serum, and misregulated inflammation. Recent toxicological evidence is limited in number and reports on the effects of Pb exposure on production of cytokines relevant to immediate-type hypersensitivity, as discussed below.

6.4.2.1 Immediate-Type Hypersensitivity

Immediate-type hypersensitivity (i.e., type I) responses are the result of the production of IgE antibodies, which trigger an array of responses, including anaphylaxis, allergic rhinitis, allergic conjunctivitis, food allergy, atopic eczema, and allergic asthma. As with other forms of hypersensitivity, immediate-type hypersensitivity develops in two stages. During the sensitization phase, antigen is presented to naive T cells by antigen-presenting cells which promotes differentiation to the Th2 phenotype and the formation of memory T cells. Memory-specific T cells interact with antigen-specific B cells leading the production of antigen-specific IgE antibodies that bind to Fc receptors on the surface of mast cells. Upon secondary exposure to the allergen, the antigen binds to mast cell-bound IgE, triggering mast cell degranulation resulting in eosinophil recruitment, mucus production, reactive airways and, potentially, anaphylaxis (Janeway et al. 2005). There are no validated animal models for determining whether a xenobiotic can cause immediate-type hypersensitivity. For that reason, the potential for a chemical to cause immediate-type hypersensitivity is assessed using a weight of the evidence approach where data from an array of experimental endpoints (total serum IgE, antigen-specific IgE, eosinophilia of the lung, measures of lung function, etc.) are carefully integrated (IPCS 2012).

As reviewed in the 2013 Pb ISA, toxicological evidence, and to a lesser extent epidemiologic evidence, have supported the effects of Pb exposure on stimulating Th2 activity. Studies have reported increased lymph node cell proliferation (Teijón et al. 2010; Carey et al. 2006), increased production of Th2 cytokines such as IL-4 (Fernandez-Cabezudo et al. 2007; Iavicoli et al. 2006; Chen et al. 2004; Heo et al. 1998; Miller et al. 1998; Heo et al. 1997; Heo et al. 1996), increased total serum IgE antibody levels (Snyder et al. 2000; Miller et al. 1998; Heo et al. 1997; Heo et al. 1996), and misregulated inflammation (Lodi et al. 2011; Chetty et al. 2005; Flohé et al. 2002; Shabani and Rabbani 2000; Miller et al. 1998; Chen et al. 1997; Knowles and Donaldson 1997; Baykov et al. 1996; Lee and Battles 1994; Zelikoff et al. 1993; Knowles and Donaldson 1990; Hilbertz et al. 1986; Castranova et al. 1980). These endpoints comprise a well-recognized mode of action for the development and exacerbation of atopic and inflammatory conditions such as asthma and allergy.

Only two recent toxicology studies investigated the effects of Pb exposure on production of cytokines relevant to immediate-type hypersensitivity. In one of these studies, administration of Pb acetate drinking water (300 ppm; BLL = 18.48 μg/dL) for 42 days decreased IFN-γ levels, but had no effect on IL-10 levels (data not shown) in adult male Sprague Dawley rats (Fang et al. 2012). In addition, administration of Pb acetate in drinking water (0.2%; BLL = 9.3 μg/dL) for 84 days had no effect on erythropoietin, GM-CSF, IL-6, and TNF-α levels in blood collected from Sprague Dawley rats (data from sexes pooled) (Cai et al. 2018). Study-specific details, including animal species, strain, sex and BLLs, are highlighted in Table 6-14.

6.4.3 Integrated Summary of Sensitization and Allergic Responses

As reviewed in the 2013 Pb ISA (U.S. EPA 2013), toxicological evidence, and to a lesser extent epidemiologic evidence, have supported the effects of Pb exposure on increased lymph node cell proliferation, increased production of Th2 cytokines such as IL-4, increased total serum IgE antibody levels in serum, and misregulated inflammation. Additionally, a limited number of longitudinal epidemiologic studies evaluated in the 2013 ISA (U.S. EPA 2013) provide evidence of associations between exposure to Pb and asthma (Joseph et al. 2005) and allergic sensitization (Jedrychowski et al. 2011). The associations in these studies are imprecise (i.e., wide 95% CIs), but are supported by cross-sectional studies of cord blood and blood Pb-associated prevalent asthma and population-based cross-sectional studies in children that reported associations between BLL and elevated serum IgE (U.S. EPA 2013). Many of these cross-sectional studies had limited adjustment for potential confounders and included populations with mean BLLs >5 μg/dL.

Though limited in number, recent PECOS-relevant animal toxicological studies continue to support the findings from the last review. Specifically, these studies consistently report effects of Pb on sensitization and allergic responses including two studies of the effects of Pb exposure on production of cytokines relevant to immediate-type hypersensitivity. In contrast, recent epidemiologic evidence is not consistent with studies evaluated in the 2013 ISA. Specifically, recent studies provide little evidence of an association between exposure to Pb and atopic disease, and inconsistent evidence for immunological biomarkers involved in hypersensitivity and allergic response. Similar to cohort studies evaluated in the 2013 ISA, recent longitudinal analyses are limited in number and have limited statistical power because of small case numbers. Among other things, limited statistical power results in the reduced likelihood of detecting a true effect and a reduced likelihood that an observed result reflects a true effect. Whereas there was coherence between the animal toxicological and epidemiologic evidence evaluated in the previous ISA, the recent evidence is less coherent given the inconsistencies and null findings across epidemiologic studies.

6.5 Autoimmunity and Autoimmune Disease

Autoimmunity is characterized by the reaction of autoreactive T lymphocytes or autoantibodies against self-molecules (i.e., autoantigens). Depending on the etiology, autoimmunity may lead to the development of autoimmune diseases such as rheumatoid arthritis and lupus. While the precipitating event for the development of autoimmunity is often unknown, intrinsic factors (e.g., gene polymorphisms, sex-related hormones, and age) and extrinsic factors (e.g., lifestyle, exposure to certain drugs, chemicals, and infectious agents) are known to play a role in the induction, development, or exacerbation of autoimmunity (IPCS 2012). Although animal models have been used to study a variety of autoimmune diseases, there are currently no validated models to assess or identify chemicals that induce or exacerbate autoimmune diseases (IPCS 2012). Consequently, the potential to induce or exacerbate autoimmunity is best investigated using a tiered approach composed of multiple methods. The 2013 Pb ISA concluded the available toxicological and epidemiologic studies were inadequate to infer that a causal relationship exists between Pb exposure and the development of autoimmunity and autoimmune disease.

6.5.1 Epidemiologic Studies of Autoimmunity and Autoimmune Disease

A single epidemiologic study evaluated in the 2013 Pb ISA (U.S. EPA 2013) examined the association between exposure to Pb and autoimmunity (El-Fawal et al. 1999). While the authors reported higher levels of autoantibodies in Pb-exposed battery workers, the analysis did not include adjustment for important confounders (e.g., other occupational exposures) and included BLLs of 10–40 µg/dL, much higher than those found in the general population. Recent epidemiologic studies of autoimmunity are limited in number and examine disparate outcomes. Mean BLL used in each study, along with other study-specific details, including study population characteristics and select effect estimates, are highlighted in Table 6-15. An overview of the recent evidence is provided below.

Two recent population-based cross-sectional studies provide inconsistent evidence of associations between exposure to Pb and autoimmune disorders (Joo et al. 2019; Kamycheva et al. 2017). In an NHANES analysis of seropositivity for Celiac Disease (i.e., tissue transglutaminase [tTg]-IgA), Kamycheva et al. (2017) reported lower adjusted mean BLLs in children with Celiac Disease compared with those without (−0.14 µg/dL [95% CI: −0.27, −0.02 µg/dL]). Associations were comparable in magnitude, but less precise in adults (i.e., wider 95% CIs). While cross-sectional studies cannot establish temporality, the nature of malabsorption in Celiac Disease makes it biologically plausible that the disorder could result in reduced absorption of Pb rather than there being a protective effect of Pb exposure. Another population-based study did not observe an association between BLL and rheumatoid arthritis (Joo et al. 2019). A notable limitation of this study is that it included children, while rheumatoid arthritis primarily affects adults.

6.5.2 Toxicological Studies of Autoimmunity and Autoimmune Disease

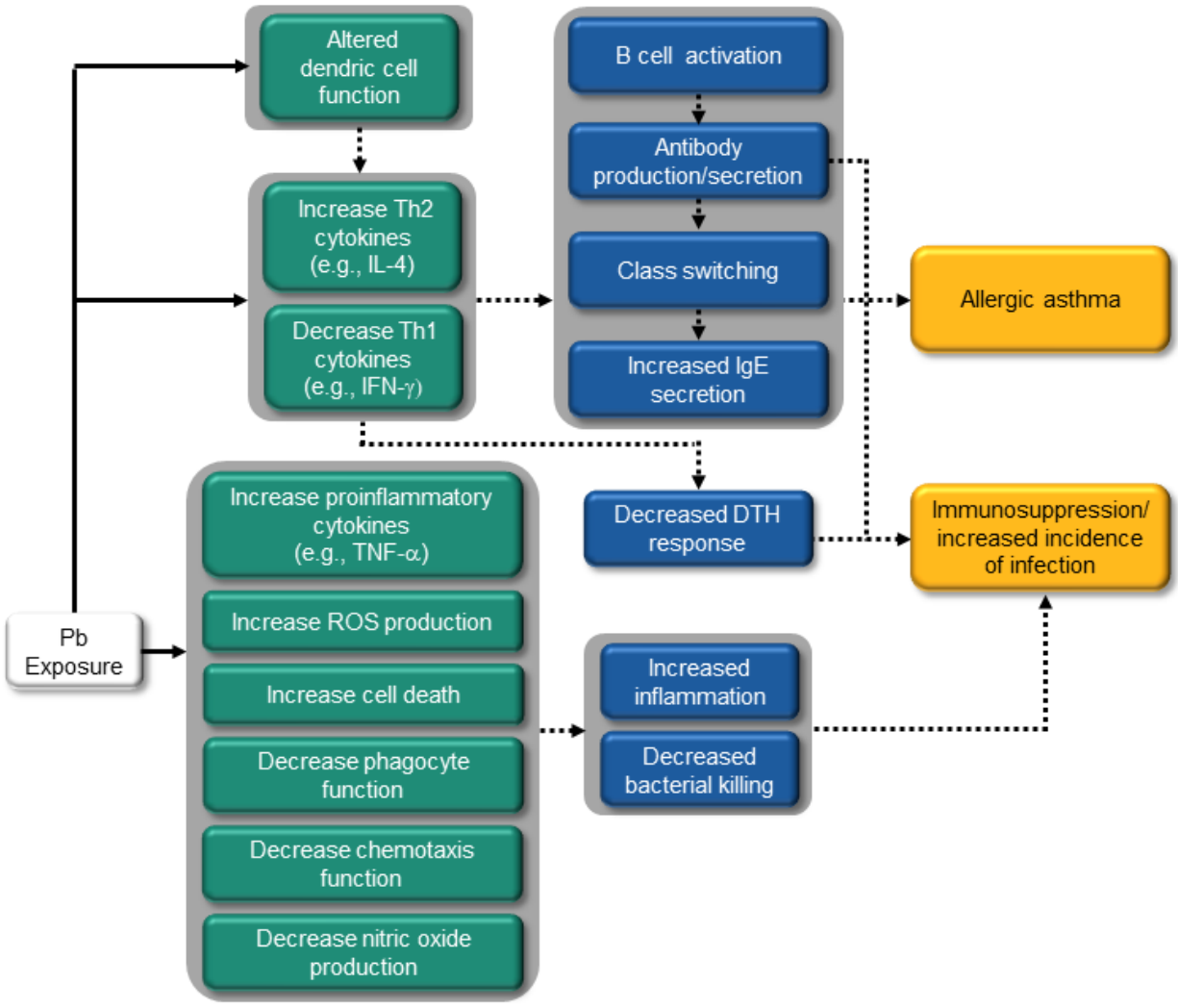
As reported in the 2013 Pb ISA, evidence for the ability of Pb to induce autoimmunity is limited (U.S. EPA 2013). Only one study performed in rats showed the generation of autoantibodies following Pb administration by a relevant route of exposure (i.e., dietary) (El-Fawal et al. 1999). Several other studies utilized Pb exposure routes or doses that produced BLLs that are not relevant to humans (Hudson et al. 2003; Bunn et al. 2000; Waterman et al. 1994). There is only one recent toxicology study that investigates an endpoint directly related to the development of autoimmunity. In that study, Fang et al. (2012) reported that administration of Pb acetate in drinking water for 42 days (BLL = 18.48 µg/dL) had no effect on the suppressive properties of Tregs isolated from adult male Sprague Dawley rats. Study-specific details, including animal species, strain, sex, and BLLs are highlighted in Table 6-16.

6.5.3 Integrated Summary of Autoimmunity and Autoimmune Disease

An epidemiologic study evaluated in the 2013 ISA (U.S. EPA 2013) observed an association between higher BLLs and elevated autoantibodies, but the strength of conclusions that can be drawn from this study is limited because it did not control for important confounders. Toxicological evidence demonstrating that Pb exposure leads to autoimmunity is similarly limited. As discussed in the previous ISA (U.S. EPA 2013), one PECOS-relevant study and several other studies utilizing non-PECOS routes of exposure and doses that produced BLLs that are not relevant to humans showed the generation of autoantibodies following Pb administration. Recent epidemiologic studies of autoimmunity are limited in number, examine disparate outcomes and provide inconsistent evidence of associations between exposure to Pb and autoimmune disorders. A recent toxicological study reported that Pb exposure had no effect on the suppressive properties of Tregs, which are critical mediators of immune tolerance.

6.6 Biological Plausibility

This section describes biological pathways that potentially underlie effects on the function of the immune system resulting from exposure to Pb. Figure 6-1 depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. Evidence supporting these proposed pathways was derived from Sections 6.3, 6.4, and 6.5 of this ISA, evidence reviewed in the 2013 ISA (U.S. EPA 2013), and recent evidence collected from studies that may not meet the current PECOS criteria, but contain mechanistic information supporting these pathways. This discussion of how exposure to Pb may lead to immune system effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in the ensuing sections. Note that the structure of the Biological Plausibility section and the role of biological plausibility in contributing to the weight-of-evidence analysis used in the 2013 Pb ISA are discussed below.



DTH = delayed-type hypersensitivity; IgE = immunoglobulin E; IFN-γ = interferon-gamma; IL-4 = interleukin 4; ROS = reactive oxygen species; Th2 = T helper; TNF-α = tumor necrosis factor alpha.

Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to Pb exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway used in an experimental study involving Pb exposure. Dotted arrows denote a possible relationship between effects. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color coded (white, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population-level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below. The structure of the biological plausibility sections and the role of biological plausibility in contributing to the weight-of-evidence analysis used in the 2022 Pb ISA are discussed in Section 6.7.

Figure 6-1 Potential biological plausibility pathways for immunological effects associated with exposure to Pb.

Immunotoxicity may be expressed as immunosuppression, unintended stimulation of immune responses, hypersensitivity, or autoimmunity (IPCS 2012). The World Health Organization's *Guidance for Immunotoxicity Risk Assessment for Chemicals* (IPCS 2012) describes best approaches for weighing immunotoxicological data. Within this framework, data from endpoints observed in the presence of

immune challenge (e.g., including effects on antibody responses, host resistance, and ex vivo WBC function) are considered most informative whereas other measures collected in the absence of immune stimulation (e.g., immune organ pathology, non-specific immunoglobulin levels, WBC counts, lymphocyte subpopulations, T cell subpopulations, immune organ weights) are considered supporting evidence. Careful review of the evidence base suggests that exposure to Pb has the potential to modulate the immune system leading to immunosuppression and sensitization and allergic responses. Below, evidence from peer-reviewed toxicology studies providing biological plausibility for Pb-associated immunotoxicity is reviewed.

6.6.1 Immunosuppression

Immunosuppression can lead to the increased incidence and severity of infectious and neoplastic diseases. Importantly, there are internationally validated animal models and human correlates (e.g., the rodent DTH assay and the human tuberculin test) for assessing the potential for a chemical to induce immunosuppression. Still, the potential for a chemical to suppress the function of the immune system is best assessed using a weight of the evidence approach where data from an array of experimental endpoints are carefully integrated (IPCS 2012).

The initiating event that ultimately leads to Pb-induced immunosuppression is unknown. However, Pb exposure has been shown to affect several indicators of immunosuppression including decreased Th1 cytokine production, production of other inflammatory mediators, decreased macrophage function (chemotaxis and phagocytosis), and ultimately suppressed the DTH response (Figure 6-1).

Exposure to Pb has been convincingly shown to result in the skewing of T cell populations, simultaneously promoting the formation of Th2 cells while suppressing the formation of Th1 cells and their cytokines including IFN-γ that play key roles in cell-mediated immunity (Heo et al. 1996; Fochtman et al. 1969). Available evidence suggests that this phenomenon may involve Pb-induced effects on dendritic cells, which promote skewing towards the Th2 phenotype (Gao et al. 2007). Mitogen-stimulated production of IFN-γ was significantly lower in splenocytes collected from Pb-exposed mice (Dvorožňáková and Jalčová 2013). IFN-γ levels in serum were reduced in Pb-exposed mice (Ajouaoi et al. 2020). IFN-γ is the primary cytokine that stimulates recruitment of macrophages associated to sites of inflammation (Lee et al. 2001; Chen et al. 1999). Relevant decrements in macrophage function associated with Pb exposure have been reported, including decreased chemotaxis (Lodi et al. 2011; Bishayi and Sengupta 2006) and phagocytosis (Lodi et al. 2011; Bussolaro et al. 2008; Bishayi and Sengupta 2006; Hilbertz et al. 1986; Zhou et al. 1985; Castranova et al. 1980). Macrophages play a vital role in cell-mediated immunity, which is often assessed using the DTH response when assaying potential immunosuppressants. Pb exposure has been consistently shown to suppress the DTH response in rodents with BLLs relevant to human exposures. Observations of a concomitant decrease in IFN-γ strengthen the link between Pb-induced inhibition of Th1 functional activities and suppression of the DTH response (Lee

et al. 2001; Chen et al. 1999). Furthermore, the effects of Pb exposure on macrophage PGE2 (Chetty et al. 2005), decreased ROS production (Chen et al. 1997; Hilbertz et al. 1986; Castranova et al. 1980), decreased NO production (Farrer et al. 2008; Mishra et al. 2006; Bunn et al. 2001b; Lee et al. 2001; Krocova et al. 2000; Chen et al. 1997; Tian and Lawrence 1996; Tian and Lawrence 1995), and increased cell death (Metryka et al. 2021; Guan et al. 2020; Choi et al. 2018; Kerr et al. 2013) may contribute to decreased resistance to bacterial or viral infection (Hilbertz et al. 1986; Castranova et al. 1980). Pb exposure has also been shown to increase levels of TNF-α, a proinflammatory cytokine, secreted by LPS-stimulated mouse J774A.1 macrophages (Luna et al. 2012) and human THP-1 monocytes through a mechanism involving ERK1/2 (Khan et al. 2011). As reviewed in the 2006 Pb AQCD (U.S. EPA 2006), Pb exposure also has the potential to reduce neutrophil chemotaxis, phagocytosis, and respiratory oxidative burst, but the effect was not judged to be as strong as what has been observed in relation to macrophages. Finally, decreased Th1 signaling leading to differences in IgG isotypes produced in response to *S. enterica* infection was implicated in impaired host defense in mice (Fernandez-Cabezudo et al. 2007).

While there is compelling evidence that Pb exposure can decrease host resistance to infection, the effect may not be attributable to direct effects of Pb exposure on the immune system. Instead, decreased host resistance may be the result of Pb acting on the microbiome. The microbiome is the body's gateway, disruption of microbiome can have profound effects on xenobiotic processing, and resistance to pathogens (Zhai et al. 2020; Dietert and Silbergeld 2015; Nriagu and Skaar 2015). The human microbiome comprises most of the cells and genes in the human body, and these cells are the first to be exposed to environmental chemicals. The microbiome plays a key role in excretion levels, transport barriers (e.g., skin, lung, gut barriers), metabolism of xenobiotics (Zhai et al. 2020; Dietert 2018; Nriagu and Skaar 2015). In addition, changes in the composition of the microbiome following exposure to xenobiotics can affect the process of colonization resistance to pathogens which may lead to loss of mucosal barrier function, elevated risk of infection, and the development of noncommunicable diseases such as asthma (Huang et al. 2020; Zhai et al. 2020; Dietert 2018; Nriagu and Skaar 2015). Importantly, Pb is known to possess antimicrobial properties(Miyano et al. 2007). As reviewed by Liu et al. (2021), exposure to Pb has been shown to alter the diversity and relative composition of the gut microbiota in several toxicology studies performed in laboratory animals. Our ability to interpret these findings is limited, however, by the fact that the investigators conducting these studies either did not measure BLL at all or, in the two studies that did, the BLL was not relevant to human exposure. In addition to toxicological studies, a limited number of epidemiologic studies reported associations between biomarkers of Pb exposure and altered gut microbiota diversity, including a birth cohort study (Sitarik et al. 2020) and a few cross-sectional analyses (Zeng et al. 2022; Eggers et al. 2019). Further, the possibility that the effects of Pb on the immune system are at least partly mediated by the microbiome is supported by the capacity of certain probiotics to protect against Pb-induced toxicity (i.e., decreases BLL and relieves Pb-induced intestinal barrier impairment) in mice (Zhai et al. 2020). In rats, chelation treatment reduced IL-4 production and IFN-γ suppression induced by Pb (Chen et al. 1999). Similarly, Vitamin D supplementation was shown to

reduce Pb-induced IL-4 in rats, but the concentration of IL-4 remained significantly elevated relative to control (BaSalamah et al. 2018).

6.6.2 Sensitization and Allergic Responses

Hypersensitivity responses (i.e., allergies) are the result of an over-reaction of the immune system. Immediate-type hypersensitivity responses are the result of the production of IgE antibodies, which trigger an array of responses including anaphylaxis, allergic rhinitis, allergic conjunctivitis, food allergy, atopic eczema, and allergic asthma. Like with other forms of hypersensitivity, immediate-type hypersensitivity, develops in two stages. During the sensitization phase, antigen is presented to naive T cells by antigen-presenting cells, which promotes differentiation to the Th2 phenotype and the formation of memory T cells. Memory-specific T cells interact with antigen-specific B cells leading the production of antigen-specific IgE antibodies that bind to Fc receptors on the surface of mast cells. Upon secondary exposure to the allergen, the antigen binds to mast cell-bound IgE, triggering mast cell degranulation resulting in eosinophil recruitment, mucus production, reactive airways and, potentially, anaphylaxis (Janeway et al. 2005). Importantly, there are no validated animal models for determining whether a xenobiotic can cause allergic asthma. For that reason, the potential for a chemical to cause allergic asthma is assessed using a weight of the evidence approach where data from an array of experimental endpoints are carefully integrated (IPCS 2012).

The initiating event that ultimately leads to allergic sensitization is called haptenation, the process where sensitizing chemical binds to endogenous proteins leading to detection by the immune system and ultimately allergic sensitization (Janeway et al. 2005). To date, there are no publications demonstrating that Pb acts as a hapten. Pb exposure, however, is associated with other hallmarks of allergic hypersensitivity and asthma including Th2 cytokine production, B cell activation, and production of IgE antibodies that are central to these responses.

Exposure to Pb resulting in BLLs relevant to humans has been convincingly shown to result in the skewing of T cell populations, simultaneously suppressing the formation of Th1 cells while promoting the formation of Th2 cells and cytokines that promote the development of allergic airway disease (Heo et al. 1996; Fochtman et al. 1969). IL-4 is a key regulator of immune responses produced by Th2 cells. This pleiotropic cytokine not only inhibits production of Th1 cytokines, but also promotes B cell activation, differentiation, proliferation and class switching leading to the production of IgE antibodies (Dietert and Piepenbrink 2006). Importantly, in most cases where Pb exposure was associated with increased IgE levels, IL-4 levels were also elevated (Snyder et al. 2000; Chen et al. 1999; Miller et al. 1998). IgE antibodies are a hallmark of immediate-type hypersensitivity responses that are responsible for inducing allergic asthma (Janeway et al. 2005). In sensitized individuals, binding of allergen to antigen-specific IgE antibodies on the surface of mast cells triggers mast cell degranulation and release histamine, leukotrienes, and cytokines, which in turn, produce the inflammatory-related effects associated with

asthma and allergy, i.e., airway responsiveness, mucus secretion, respiratory symptoms (Janeway et al. 2005). Consistent with this condition, inflammation was identified as a major immune-related effect of Pb based on consistent toxicological evidence for Pb-induced increases in proinflammatory cytokines (e.g., IL-4) and increased levels of PGE2 (Chetty et al. 2005) and ROS production (Chen et al. 1997; Hilbertz et al. 1986; Castranova et al. 1980), decreased NO production (Farrer et al. 2008; Mishra et al. 2006; Bunn et al. 2001b; Lee et al. 2001; Krocova et al. 2000; Chen et al. 1997; Tian and Lawrence 1996; Tian and Lawrence 1995), and increased cell death (Metryka et al. 2021; Guan et al. 2020; Choi et al. 2018; Kerr et al. 2013) that may also contribute to Pb-induced decreased resistance to bacterial or viral infection (Hilbertz et al. 1986; Castranova et al. 1980).

6.7 Summary and Causality Determination

The body of epidemiologic and toxicological evidence describes several effects of Pb exposure on the immune system. The majority of this evidence predates this ISA. These effects can be traced back to two major targets including T cells and macrophages promoting immunosuppression and sensitization and allergic responses, respectively. In addition, a very limited number of studies report findings related to autoimmunity. The sections that follow describe the evaluation of evidence for these three groups of outcomes with respect to causality determinations for exposure to Pb using the framework described in the Preamble to the ISA (U.S. EPA 2015). The key evidence, as it relates to the causal framework, is outlined below, and summarized in Table 6-1, Table 6-2, and Table 6-3.

6.7.1 Causality Determination for Immunosuppression

The 2013 Pb ISA concluded that "that a causal relationship is likely to exist between Pb exposures and decreased host resistance."(U.S. EPA 2013). This causality determination was primarily based on consistent evidence that exposure to relevant BLLs suppresses the DTH response and increases bacterial titers and subsequent mortality in rodents. For example, various studies reported decreased clearance of bacteria and increased mortality induced by *Listeria monocytogenes* in mice exposed postnatally to Pb acetate in drinking water for 3 to 8 weeks, resulting in BLL ranging from 20–25 µg/dL (Fernandez-Cabezudo et al. 2007; Dyatlov and Lawrence 2002; Kim and Lawrence 2000; Kishikawa et al. 1997; Lawrence 1981). Other studies reported increased mortality from *Salmonella* or *E. coli*, or decreased clearance of *Staphylococcus*, in mice administered Pb acetate or Pb nitrate via injection resulting in BLL relevant to the 2013 Pb ISA (Bishayi and Sengupta 2006; Cook et al. 1975; Hemphill et al. 1971; Selye et al. 1966). Although BLLs were high (i.e., 71–313 µg/dL), increased mortality from viral infection was also reported in mice and chickens administered Pb (mostly Pb acetate) for 4–10 weeks (Gupta et al. 2002; Exon et al. 1979; Thind and Khan 1978). Additional evidence for Pb-induced immunosuppression comes from studies investigating the DTH response. Suppressed DTH response is one of the most consistently reported immune effects associated with Pb exposure in animals

(U.S. EPA 2013). Suppression of the DTH response has been reported following gestational (Chen et al. 2004; Bunn et al. 2001a; Bunn et al. 2001b; Bunn et al. 2001c; Lee et al. 2001; Chen et al. 1999; Miller et al. 1998; Faith et al. 1979) and postnatal (McCabe et al. 1999; Laschi-Loquerie et al. 1984; Müller et al. 1977) exposures to Pb acetate resulting in BLLs ranging from 6.75 to >100 μg/dL) in rats, mice and chickens (U.S. EPA 2013). Further, evidence suggested a plausible mode of action involving suppressed production of Th1 cytokines (e.g., IFN-γ) (Fernandez-Cabezudo et al. 2007; Lara-Tejero and Pamer 2004), and decreased macrophage function (Lodi et al. 2011; Bishayi and Sengupta 2006; Chen et al. 1997; Hilbertz et al. 1986; Castranova et al. 1980). A limited number of epidemiologic studies reviewed in the 2013 ISA (U.S. EPA 2013) indicated an association between BLL and viral and bacterial infections in children. None of the studies considered potential confounders, however, and most analyzed populations with higher BLLs (means >10 μg/dL). Cross-sectional studies of cell-mediated immunity reported consistent associations between BLL and lower T cell abundance in children, while results from other studies on lymphocyte activation, macrophages, neutrophils, and NK cells were generally inconsistent or not sufficiently informative (e.g., cross-sectional study designs with limited or no consideration of potential confounding, and a lack of information on concentration-response relationship).

Recent toxicological studies provide additional evidence for immunosuppression. Although there were no recent studies directly investigating the effects of Pb exposure on host resistance, the ability of Pb to alter antibody responses was investigated and provides evidence for immunosuppression. Yathapu et al. (2020) showed that serum levels of anti-TT specific IgM antibodies were decreased while anti-TT specific IgG levels were unaffected in rats exposed to Pb (BLL = 16.1 μg/dL) in drinking water. Consistent with the previous ISA, administration of Pb acetate in drinking water for 42 days (BLL = 18.48 μg/dL) significantly suppressed the DTH response in adult male Sprague Dawley rats (Fang et al. 2012). Additional supporting evidence for Pb-induced immunosuppression can be derived from observational endpoints including (1) reduced non-specific mucosal IgA immunoglobulins (but not IgM or IgG) in rats with BLLs of 16.1 μg/dL (Yathapu et al. 2020) and (2) reduced relative thymus weight in juvenile rats orally administered Pb (1 or 10 mg/kg with BLL of 3.27 μg/dL and 12.5 μg/dL, respectively) for up to 25 days (Graham et al. 2011). Because of differences in experimental design parameters and specific endpoints measured, effects of Pb exposure on immune organ pathology, WBC counts and differentials, and WBC counts (hematology and subpopulations) are challenging to interpret and, for that reason, do not support or refute evidence obtained from immune function assays.

The relationship between Pb exposure and immunosuppression is further supported by recent epidemiologic studies, which expand quantity and quality of the observational evidence base evaluated in the previous ISA. Recent case-control and cross-sectional studies provide consistent evidence that BLLs are associated with increased susceptibility to viral and bacterial infection in children and adults (Feiler et al. 2020; Park et al. 2020; Krueger and Wade 2016) and reduced antibiotic resistance in children, as measured by nasal *Staphylococcus aureus* colonization (Eggers et al. 2018). Associations were observed with mean, median, or geometric mean BLLs <3.5 μg/dL. The evaluated studies used concurrent blood Pb measures, raising uncertainty regarding the temporal sequence between Pb exposure and

immunosuppression and the magnitude, timing, frequency, and duration of Pb exposures that contributed to the observed associations. Recent studies also provide generally consistent evidence of Pb-related decreases in vaccine antibodies in children with low mean or median BLLs, including a birth cohort of vaccinated children in South Africa with median BLLs <2 µg/dL Di Lenardo et al. (2020). A strength of this analysis is that it establishes temporality between exposure and outcome. Cross-sectional studies, including a large analysis of children ages 6 to 17 from the 1990-2004 NHANES (Jusko et al. 2019), are consistent with results from the prospective birth cohort. Notably, this study includes many children who were born before the phaseout of leaded gasoline and were likely subject to higher past exposures. Thus, there is uncertainty concerning the specific Pb exposure level, timing, frequency, and duration contributing to the associations observed in this study.

In summary, there is coherent and consistent evidence across toxicological and epidemiologic studies that Pb exposure induces immunosuppression leading to decreased host resistance to infection. Notably, epidemiologic studies of viral and bacterial infection used concurrent blood Pb measures, raising uncertainty regarding the temporal sequence between Pb exposure and immunosuppression and the magnitude, timing, frequency, and duration of Pb exposures that contributed to the observed associations. Furthermore, there is consistent toxicological evidence that Pb exposure suppresses the DTH response in animals. A limited body of epidemiologic studies provide consistent evidence that prenatal (mean <4 µg/dL) and concurrent (mean and/or medians <2 µg/dL) BLLs are associated with a decrease in vaccine antibody response. However, results obtained from studies investigating the TDAR to sheep red blood cells, the animal correlate for the vaccine response, were inconsistent with one study reporting a decrease it the response (BLL = 25.9) (Blakley and Archer 1981) and another investigating showing no effect in mice with high BLL (mean range 59-132 µg/dL) (Mudzinski et al. 1986). Recognizing the variety of study designs employed, the variety of endpoints assessed, the lack of replication, data from observational immune endpoints are of limited value for this assessment. Biological plausibility for the observed associations is provided by toxicological and epidemiologic studies demonstrating (1) skewing of T cell populations, promoting Th2 cell formation and cytokine production, (2) decreased IFN-γ production, (3) decrements in macrophage function, (4) production of inflammatory mediators, and (5) disruption of the microbiome. **Collectively, there is sufficient evidence to conclude that there is *likely to be a causal relationship* between Pb exposure and immunosuppression**.

Table 6-1 Summary of evidence for a likely to be causal relationship between Pb exposure and immunosuppression.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Pb Biomarker Levels Associated with Effects[c]
Consistent evidence from toxicological studies with relevant exposures investigating immune functional endpoints	Dietary Pb exposures increased bacterial infection. Similar observations in several other studies using non-PECOS routes of exposure and/or higher Pb exposures	Dyatlov and Lawrence (2002) Fernandez-Cabezudo et al. (2007)	Mean BLL: 20 µg/dL after adult 16-wk exposure 25 µg/dL after lactational exposure
	Dietary gestational Pb exposures suppressed DTH response. Similar observations in several other studies with higher Pb exposures	Chen et al. (2004) Bunn et al. (2001a) Fang et al. (2012)	Mean BLL: 6.75 µg/dL 25 µg/dL BLL = 18.48 µg/dL
Evidence from other toxicological studies with relevant exposures investigating immune functional endpoints	Pb exposure decreased levels of anti-TT-specific IgM, levels of anti-TT-specific IgG were unaffected	Yathapu et al. (2020)	Mean BLL: 16.1 ± 5.5 µg/dL
Supporting evidence from toxicological studies with relevant exposures supporting immune functional endpoints	Pb exposure decreased non-specific mucosal IgA immunoglobulins	Yathapu et al. (2020)	Mean BLL: 16.1 ± 5.5 µg/dL
	Oral administration of Pb decreased relative thymus weight in juvenile rats	Graham et al. (2011)	1 or 10 mg/kg with BLL of 3.27 µg/dL and 12.5 µg/dL, respectively
Coherence from a small body of epidemiologic studies demonstrating consistent evidence of decreased host resistance at low BLLs	A limited number of case-control and cross-sectional studies reported associations between concurrent BLLs and: Increased susceptibility to viral and bacterial infection, and	Krueger and Wade (2016)	

		Park et al. (2020) Feiler et al. (2020)	Mean, Median, or Geometric Mean BLL across studies: 1.4-3.15 µg/dL
	Reduced antibiotic resistance	Eggers et al. (2018)	
	Uncertainty regarding the temporal sequence between Pb exposure and immunosuppression and the magnitude, timing, frequency, and duration of Pb exposures that contributed to the observed associations.		
Coherence from a small body of epidemiologic studies demonstrating consistent evidence of decreased vaccine antibody response at low BLLs	A limited number of prospective birth cohort and cross-sectional studies reported associations between BLLs and decreased vaccine antibody response	Di Lenardo et al. (2020) Jusko et al. (2019) See Section 6.3.1.2	Median BLL: 1.9 µg/dL Mean BLL: 1.4 µg/dL
Biological Plausibility	Evidence that Pb (1) suppressed production of Th1 cytokines, (2) decreased macrophage function, and (3) increased inflammation in animals	See Section 6.6	

anti-TT = anti-tetanus toxoid; BLL = blood lead level; DTH = delayed-type hypersensitivity; IgG = immunoglobulin G; IgM = immunoglobulin M; Pb = lead; PECOS = population, exposure, comparator, outcome and study.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA 2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the Pb biomarker levels at which the evidence is substantiated.

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6.7.2 Causality Determination for Sensitization and Allergic Responses

The 2013 Pb ISA concluded "that a causal relationship is likely to exist between Pb exposures and an increase in atopic and inflammatory conditions."(U.S. EPA 2013). This causality determination was made on the basis of a body of evidence integrated across epidemiologic and toxicological studies. Epidemiologic evidence included a prospective analysis reporting associations between BLLs and asthma incidence in children (Joseph et al. 2005) and another longitudinal study that observed an association between cord BLLs and immediate-type allergic responses in children that were detected clinically using SPTs (Jedrychowski et al. 2011). Both studies had small sample sizes, however, and lacked precision (i.e., had wide 95% CIs), which increases the likelihood of chance findings. An additional prospective cohort analysis reported an imprecise association between cord BLLs and prevalent asthma in children (Rabinowitz et al. 1990) but did not adjust for potential confounders. The associations observed in the prospective analyses are supported by a cross-sectional study of BLL-associated parental-reported asthma in children and population-based cross-sectional studies in children that reported associations between BLL and elevated serum IgE. Notably, many of the serum IgE studies had limited adjustment for potential confounders and included population mean BLLs >5 µg/dL. The epidemiologic findings are coherent with a large body of toxicological studies that reported physiological responses in animals consistent with the development of allergic sensitization, including increased lymph node cell proliferation (Teijón et al. 2010; Carey et al. 2006), increased production of Th2 cytokines such as IL-4 (Fernandez-Cabezudo et al. 2007; Iavicoli et al. 2006; Chen et al. 2004; Heo et al. 1998; Miller et al. 1998; Heo et al. 1997; Heo et al. 1996), increased total serum IgE antibody levels (Snyder et al. 2000; Miller et al. 1998; Heo et al. 1997; Heo et al. 1996), and misregulated inflammation (Lodi et al. 2011; Chetty et al. 2005; Flohé et al. 2002; Shabani and Rabbani 2000; Miller et al. 1998; Chen et al. 1997; Knowles and Donaldson 1997; Baykov et al. 1996; Lee and Battles 1994; Zelikoff et al. 1993; Knowles and Donaldson 1990; Hilbertz et al. 1986; Castranova et al. 1980).

There have been several recent epidemiologic studies of sensitization and allergic response, including prospective birth cohorts and cross-sectional studies with mean or median BLLs <2 µg/dL. In contrast to evidence presented in the previous ISA (U.S. EPA 2013), the recent studies provide little evidence of an association between exposure to Pb and atopic disease, and inconsistent evidence for immunological biomarkers involved in sensitization and allergic response. Specifically, recent epidemiologic studies of atopic disease, including analyses of prospective cohort studies examining of asthma (Pesce et al. 2021), eczema (Pesce et al. 2021; Kim et al. 2019; Kim et al. 2013), and food allergies (Pesce et al. 2021) were generally consistent in reporting a lack of an association with low BLLs. A considerable uncertainty in the evidence base is the limited number of children with asthma in the cohort studies evaluated, both in recent studies and in the previous ISA. This decreases the statistical power to detect an association and increases the likelihood of chance findings. Notably, recent cross-sectional NHANES analyses also reported null associations between childrens' BLLs and asthma (Wells

et al. 2014), eczema (Wei et al. 2019), and food allergies (Mener et al. 2015) in much larger study populations. Results from recent epidemiologic studies of allergen-specific and non-specific immunological biomarkers of hypersensitivity in children and adults were less consistent than the generally null results for atopic diseases, providing inconsistent evidence in both children and adults.

Recent toxicological evidence for effects of Pb exposure on biomarkers of allergic disease is sparse and limited to two reports investigating cytokine levels in blood. Decreased IFN-γ, a Th1 cytokine known to play a role in the resolution of asthma, was reported in a recent study. Pb exposure had no effect on the levels of other cytokines that have been reported to play a role in allergic disease (i.e., GM-CSF, IL-6, IL-10, and TNF-α). However, the value of these data for hazard identification is limited by two factors. Changes in cytokine levels (particularly when measured in blood) can be associated with many different types of tissues and toxicities and may reflect an immune response to tissue injury but not necessarily an effect on or impairment of immune function. For this reason, cytokine secretion data (in the absence of a stimulus) are considered supporting evidence for understanding mechanisms of immune disruption, not as apical data. In addition, the utility of these data is further diminished by the lack of additional studies corroborating these findings.

In summary, recent epidemiologic studies provide little evidence of an association between exposure to Pb and atopic disease and inconsistent evidence for immunological biomarkers involved in sensitization and allergic response. However, there is consistent toxicological evidence that exposure to Pb increased lymph node cell proliferation, increased production of Th2 cytokines such as IL-4, increased total serum IgE antibody levels in serum, and misregulated inflammation in studies reporting BLL relevant to this ISA. Biological plausibility for the observed associations is provided by toxicological evidence that Pb (1) promotes the production of Th2 cells and cytokines including IL-4 and (2) increased total serum IgE levels in studies utilizing non-relevant routes of administration (i.e., injection) and in studies either reporting high BLL or those not reporting BLL at all. **Collectively, the body of evidence is *suggestive of, but not sufficient to infer, a causal relationship* between Pb exposure and sensitization and allergic responses**.

Table 6-2 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between Pb exposure and sensitization and allergic responses.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Pb Biomarker Levels Associated with Effects[c]
Consistent evidence from other toxicological studies with relevant exposures investigating immune functional endpoints	Increased IL-4 production, decreased IFN-γ production in mice administered Pb in drinking water for 16 wk	Fernandez-Cabezudo et al. (2007)	Mean BLL: 5 or 10 mM with BLL of 20.5 and 106.2 µg/dL, respectively
	Increased IL-4 production in mice exposed prenatally and postnatally	Iavicoli et al. (2006)	0.02, 0.06, 0.11, 0.2, 40.00, and 400.0 ppm with mean BLL of 0.83, 1.23, 1.59, 1.97, 11.86, and 61.48 µg/dL, respectively
	Increased total serum IgE antibody in mice exposed prenatally and postnatally to 0.1 mM Pb acetate for 2 wk	Snyder et al. (2000)	Mean BLL: 25.3 µg/dL
Inconsistent epidemiologic evidence for atopic disease provides limited coherence with toxicological evidence	A limited number of studies reported positive but imprecise associations between BLLs and asthma incidence and prevalence in children. Studies limited by small number of cases	Joseph et al. (2005) Pugh Smith and Nriagu (2011)	Associations observed in stratified analysis for participants with BLLs ≥5 and ≥10 µg/dL
	A limited number of recent studies with lower BLLs reported null associations between BLLs and asthma incidence and prevalence in children	Pesce et al. (2021) Wells et al. (2014)	Mean cord BLL: 1.45 µg/dL Geometric Mean BLL: 1.13 µg/dL
	Generally null associations observed in studies of other atopic diseases in children, including eczema and food allergies	See Section 6.4.2	Mean/Median BLL across studies: 1.01–1.75 µg/dL
Biological Plausibility	Evidence that Pb (1) promotes T cell skewing leading to the production of Th2 cells and cytokines including IL-4, (2) increased IgE levels, and (3) increased inflammation in animals	See Section 6.6	

BLL = blood lead level; IFN-γ = interferon-gamma; IgE = immunoglobulin E; IL-4 = interleukin 4; Pb = lead.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA 2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the Pb biomarker levels at which the evidence is substantiated.

6.7.3 Causality Determination for Autoimmunity and Autoimmune Disease

In the 2013 Pb ISA, it was concluded that "that the evidence is inadequate to determine if there is a causal relationship between Pb exposure and autoimmunity." (U.S. EPA 2013). This causality determination was reached based on evaluation of a limited body of evidence that does not sufficiently inform Pb-induced generation of autoantibodies with relevant Pb exposures. While elevated levels of autoantibodies were reported in a single study of Pb-exposed battery workers (El-Fawal et al. 1999), the internal validity and relevance of this study to this ISA is uncertain because of a lack of adjustment for important confounders and a study population with BLLs (10–40 μg/dL) that are much higher than those found in the general population. In the only toxicology study available for the 2013 Pb ISA with BLLs relevant to humans, autoantibodies were detected in rats following dietary administration of Pb resulting in BLLs of 11–50 μg/dL (El-Fawal et al. 1999).

Recent epidemiologic studies of autoimmunity are limited in number and examine disparate outcomes (Joo et al. 2019; Kamycheva et al. 2017). Neither study observed evidence supporting an association between Pb exposure and autoimmunity. Although Kamycheva et al. (2017) reported an inverse association between BLLs and seropositivity for Celiac Disease, the cross-sectional study design does not preclude reverse causality, whereby the association may result from reduced absorption of Pb rather than a protective effect of Pb exposure. Only one recent toxicology study was available for this assessment. In that study, Fang et al. (2012) reported that administration of Pb acetate in drinking water for 42 days (BLL = 18.48 μg/dL) had no effect on the suppressive properties of Tregs isolated from adult male Sprague Dawley rats. Recent studies do not indicate a relationship between exposure to Pb and autoimmunity and the limited number of studies and disparate outcomes examined make it difficult to draw conclusions about the nature of the relationship. **Therefore, the body of evidence remains *inadequate to infer the presence or absence of a causal relationship* between exposure to Pb and autoimmunity.**

Table 6-3 Summary of evidence that is inadequate to determine if a causal relationship exists between Pb exposure and autoimmunity and autoimmune disease.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Pb Biomarker Levels Associated with Effects[c]
Limited toxicological evidence for increased autoantibodies	A study in rats shows generation of autoantibodies with relevant adult-only dietary Pb exposure for 4 d. Several other studies have Pb exposure concentrations and/or exposure routes (e.g., intraperitoneal) with uncertain relevance to humans	El-Fawal et al. (1999)	BLL: 11–50 µg/dL
Coherence from a limited number of epidemiologic studies for increased autoantibodies at high BLLs	Evidence for increased autoantibodies in Pb-exposed workers with high BLL and limited consideration for potential confounding, including other workplace exposures	El-Fawal et al. (1999)	BLL: 10–40 µg/dL
Lack of coherence from epidemiologic studies of autoimmune disease	Limited number of epidemiologic studies reported null or associations between BLLs and	Kamycheva et al. (2017) Joo et al. (2019)	
Limited evidence for biological plausibility	Administration of Pb for 42 d had no effect on Treg activity in rats	Fang et al. (2012)	BLL: 18.48 µg/dL

BLL = blood lead level; d = day; Pb = lead; Treg = regulatory T cells.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA 2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the Pb biomarker levels at which the evidence is substantiated.

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6.8 Evidence Inventories – Data Tables to Summarize Study Details

Table 6-4 Epidemiologic studies of exposure to Pb and immunosuppression.

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
Host Resistance					
[†]Eggers et al. (2018) United States 2001–2004 Cross-Sectional	NHANES n: 18626 General population; ≥1 yr old	Blood Blood Pb was measured in venous whole blood using GFAAS (2001–2002) and ICP-MS (2003–2004) Age at measurement: ≥1 yr old Median: 1.4 µg/dL 75th: 2.3 µg/dL Maximum: 68.9 µg/dL Q1: <0.91 µg/dL Q2: 0.91–1.4 µg/dL Q3: 1.41–2.3 µg/dL Q4: >2.3 µg/dL	Prevalence of MRSA and MSSA colonization Colonization by *S. aureus* tested using nasal swabs and standard culture-based procedures Age at Outcome: ≥1 yr old	Age, sex, race, income, smoking, iron, calcium, and Vitamin C	**ORs** **MRSA Colonization:** Q1: Reference Q2: 1.52 (0.83, 2.76) Q3: 1.56 (0.75, 3.24) Q4: 1.82 (0.81, 4.1) **MRSA Colonization:** Q1: Reference Q2: 1.07 (0.95, 1.21) Q3: 1.1 (0.94, 1.28) Q4: 0.91 (0.76, 1.09)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Krueger and Wade (2016) United States 1999-2012 Cross-Sectional	NHANES n: 18,425 (*T. gondii*) 17,389 (Hepatitis B), 5,994 (*H. Pylori*) General population; ≥3 yr old (*H. Pylori*), ≥6 yr old (*T. gondii* and HBV)	Blood Blood Pb was measured in venous whole blood using ICP-MS Age at measurement: >3 yr old (*H. Pylori*), ≥6 yr old (*T. gondii* and HBV) Geometric mean: 1.5 µg/dL	Seropositivity for *T. gondii*, *H. Pylori*, and Hepatitis B Serum tested for *T. gondii* and *H. Pylori* IgG antibodies using an ELISA and HBc ELISA was used to detect total antibodies against Hepatitis B core antigen Age at Outcome: ≥3 yr old (*H. Pylori*), ≥6 yr old (*T. gondii* and HBV)	Age, sex, race/ethnicity, country of birth, family income, self-reported health, tap water source, household crowding, NHANES cycle, and use of illicit intravenous drugs	**ORs** ***H. Pylori* Seropositivity**: 1.09 (1.05, 1.13) ***T. Gondii* Seropositivity**: 1.10 (1.06, 1.14) **Hepatitis B Seropositivity**: 1.08 (1.03, 1.13)
†Feiler et al. (2020) Rochester, NY United States 2012-2017 Case-control	n: 2,663 (full sample); 617 (reduced sample) Test-negative case-control study of children <4 yr old tested for influenza/RSV	Blood Blood Pb measured in venous or capillary whole blood samples using GFAAS. When multiple measurements were available Age at measurement: Between 6 mo and 4 yr Mean: NR ~60% of children had peak BLLs <1 µg/dL; 5% had peak BLLs >5 µg/dL	Influenza and RSV diagnosis Nasopharyngeal swab samples tested for influenza or RSV by PCR Age at Outcome: <4 yr old	Full sample: age, sex, race, ethnicity, insurance status, and respiratory season. Reduced sample: Same as full, plus maternal age, parity, feeding type, maternal smoking, and area-level poverty, unemployment, education, and housing built before 1980	**ORs** **Influenza** **<1 µg/dL:** Reference **1-3:** 1.52 (0.69, 3.37) **>3:** 1.12 (0.45, 2.82) **RSV** **<1 µg/dL:** Reference **1-3:** 0.97 (0.56, 1.66) **>3:** 0.9 (0.5, 1.62)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Park et al. (2020) Hwasun South Korea 2014-2016 Cross-sectional	n: 2625 Patients ≥20 yr old undergoing gastrointestinal endoscopy	Blood Blood Pb measured in whole blood using GFAAS Age at measurement: ≥20 yr old Mean: Men: 3.15 µg/dL; Women: 2.19 µg/dL	*H. Pylori* infection *H. Pylori* infection confirmed histologic examination using Giemsa staining of abnormal lesions identified during endoscopy Age at Outcome: ≥20 yr old	Age, smoking, drinking, BMI, and diabetes, exercise	**ORs** ***H. Pylori* Infection** **Men:** 1.05 (1.03, 1.08) **Women:** 1.06 (1.00, 1.13)
Vaccine Antibody Response					
†Di Lenardo et al. (2020) Limpopo South Africa 2012-2013 Cohort	Venda Health Examination of Mothers, Babies and their Environment n: 425 Women recruited when presenting for delivery. Children were excluded if they did not receive measles, tetanus, and Hib immunizations	Blood Blood Pb measured in triplicate in whole blood using ICP-MS Age at measurement: 1 yr Median: 1.9 µg/dL 75th: 2.8 µg/dL	Measles, Tetanus, and *H. influenzae* type B IgG titers Serum IgG specific to measles, tetanus, and Hib measured by ELISA Age at Outcome: 3.5 yr	Maternal age, HIV status, duration of breast feeding	**ORs for odds of being below protective cut point** **Measles IgG levels**: 1.00 (0.77, 1.31) **Tetanus IgG levels:** 1.13 (1.02, 1.26) **Hib IgG levels:** 0.99 (0.89, 1.11)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Jusko et al. (2019) United States 1999-2004 Cross-Sectional	NHANES n: 7005 General population; children 6–17 yr old. Percent unvaccinated not reported. MMR vaccine schedule between 1999 and 2004 was: 1st dose: 12–18 mo; 2nd dose: 4–6 yr; and Catch-up 2nd dose by 11–12 yr	Blood Blood Pb was measured in venous whole blood using ICP-MS Age at measurement: 6–17 yr old Mean: 1.4 µg/dL Median: 1.0 µg/dL	Measles, Mumps, and Rubella Antibody Levels Measles and Rubella antigen-specific IgG levels were determined using an ELISA; Mumps antigen-specific IgG levels were determined via Wampole Mumps IgG test Age at Outcome: 6–17 yr old	Sex, age, race/ethnicity, family poverty-income ratio, and NHANES cycle	**% Change** **Anti-Measles IgG levels:** −2.75 (−5.10, −0.41) **Anti-Mumps IgG levels:** −2.07 (−3.87, −0.24) **Anti-Rubella IgG levels:** 0.00 (−2.58, 2.65)
†Welch et al. (2020) Munshiganj and Pabna Bangladesh 2008-2011 enrollment (follow-up through 5 yr of age) Cohort	n: 502 Pregnant women with singleton pregnancies recruited and children followed through 5 yr of age	Blood Cord blood Pb measured using ICP-MS; Blood Pb measure in capillary samples using portable Lead-Care II instruments Age at measurement: At birth, 20–40 mo and 4-5 yr Median: Pregnancy: 3.1 µg/dL; Toddler: 6.4 µg/dL; Early Childhood: 4.7 µg/dL 75th: Pregnancy: 5.6 µg/dL; Toddler: 10.0 µg/dL; Early Childhood: 7.0 µg/dL	Serum vaccine antibody concentrations (diphtheria and tetanus) Serum diphtheria and tetanus antibodies measured using an ELISA Age at Outcome: 5 yr old	Maternal education, breastfeeding duration, and child sex	**% Change in Median Antibody Concentration** *Cord BLLs* **Diphtheria:** 0.97 (−1.11, 3.05) **Tetanus:** 1.54 (−0.17, 3.24) *BLLs* **Diphtheria:** −0.96 (−3.26, 1.33) **Tetanus:** 0.33 (−2.36, 3.02)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Xu et al. (2015) Shantou China 2011-2013 Cross-sectional	n: 490 Hepatitis B vaccinated children 3–7 yr old from two kindergartens (one near an e-waste facility, and the other in a matched reference area)	Blood Blood Pb measured in venous whole blood using GFAAS Age at measurement: 3–7 yr old Geometric Mean: Reference kindergarten: 6.05 µg/dL; Exposed (e-waste) kindergarten: 6.76 µg/dL	Hepatitis B surface antibody levels Blood plasma HBsAb titer was measured by ELISA Age at Outcome: 3–7 yr old	Age and sex (areas matched on traffic density, population, SES, lifestyle, and cultural background)	**Change in HBsAb titers (S/CO)** **2011 Sample:** -0.45 (-0.49, −0.40) **2012 Sample:** -0.37 (-0.40, −0.33)
WBCs and Cytokines					
†Cao et al. (2018) Guiyu and Haojiang China 2014 Cross-Sectional	n: 118 Children 3–7 yr old at two kindergartens (one near an e-waste facility, and the other in a matched reference area)	Blood Pb measured in venous whole blood using GFAAS Age at measurement: 3–7 yr Median: Reference kindergarten: 3.6 µg/dL Exposed (e-waste) kindergarten: 5.1 µg/dL	T cell subpopulations, IL-2, IL-7, IL-15 levels T cell subpopulations measured in whole blood using flow cytometry; Serum cytokines measured using the ProcartaPlex Human Cytokine Chemokine Panel 1A Age at Outcome: 3–7 yr	Age and sex (areas matched on traffic density, population, SES, lifestyle, and cultural background)	**Change in percentage of T cells** **CD4+ Tn** -0.59 (-1.07, -0.12) **CD4+ Tcm** 0.49 (0.10, 0.88)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Chen et al. (2021) Shantou China Nov.-Dec. 2018 Cross-sectional	n: 486 Pre-school children (aged 2–6) from two towns with similar SES but different Pb exposure	Blood Blood Pb measured in venous whole blood using GFAAS Age at measurement: 2–6 yr Median: Exposed: 4.51 µg/dL; Reference: 3.98 µg/dL 75th: Exposed: 5.67 µg/dL, Reference: 4.84 µg/dL	WBC, neutrophil, and monocyte counts WBCs, neutrophils, and monocytes measured in venous whole blood Age at Outcome: 2–6 yr	Gender, age, BMI, e-waste contamination w/ in 50 m of residence, residence as workplace, distance of residence from road, family member daily smoking, monthly household income, maternal work associated w/ e-waste, duration of outdoor play, child contact w/ e-waste, washing hands before eating, nail biting habit, chewing pencil habit, yearly canned food consumption, yearly fruit/vegetable consumption, yearly iron rich food consumption, yearly marine product consumption, and yearly salted food consumption	**ln(WBC count)** 0.006 (0.001, 0.012) **ln(Monocyte count)** 0.006 (-0.001, 0.013) **ln(Neutrophil count)** 0.009 (0, 0.018)
†Dai et al. (2017) Shantou China Cross-sectional	n: 484 Children 2–6 yr old randomly sampled from volunteers at two kindergartens (one near an e-waste facility, and the other in a matched reference area)	Blood Blood Pb measured in venous whole blood using GFAAS Age at measurement: 2–6 yr old Q1: <3.78 µg/dL Q2: 3.78–5.22 µg/dL Q3: 5.23–7.00 µg/dL Q4: >7.00 µg/dL	Erythrocyte CR1 expression measured using flow cytometry Age at Outcome: 2–6 yr old	Age, gender, paternal and maternal education level, and family income	**Mean Difference in Erythrocyte CR1 Expression** **Q1:** Reference **Q2:** −0.07 (−0.23, 0.08) **Q3:** −0.04 (−0.20, 0.11) **Q4:** −0.16 (−0.32, −0.01)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Huo et al. (2019) Shantou China NR Cross-sectional	n: 267 Children 2–7 yr old at two kindergartens (one near an e-waste facility, and the other in a matched reference area)	Blood Blood Pb measured in venous whole blood using GFAAS Age at measurement: 2–7 yr old Median: Reference kindergarten: 4.4 µg/dL; Exposed (e-waste) kindergarten: 6.5 µg/dL 75th: Reference kindergarten: 5.6 µg/dL; Exposed (e-waste) kindergarten: 8.2 µg/dL	IFN-γ, IL-1ß, and IL-12p70 <U+03B3> Serum cytokine measured using the ProcartaPlex Human Cytokine Chemokine Panel 1A Age at Outcome: 2–7 yr old	Age and sex (areas matched on traffic density, population, SES, lifestyle, and cultural background)	Per natural log increase in erythrocyte Pb **IL-1β pg/ml** 0.08 (-0.01, 0.17) **IL-12p70 pg/ml** 0.99 (0.53, 1.44) **IFN-γ pg/ml** 1.43 (0.57, 2.30)
†Li et al. (2018) Hubei and Hunan Provinces China 2012–2017 Cross-Sectional	Blood Lead Intervention Program n: 758 Children Ages 5–8 yr recruited from 4 counties in 2 provinces. One county in each province had high environmental Pb levels (battery plant and mining)	Blood Blood Pb measured in venous whole blood using GFAAS Age at measurement: 5–8 yr old Geometric mean: 8.24 µg/dL 75th: 13.51 µg/dL 90th: 18.77 µg/dL 95th: 21.82 µg/dL	WBC count Hematological parameters were analyzed by an automated hematology analyzer (BC-5800; Mindray, Shenzhen, China) with quality control processes. Age at Outcome: 5–8 yr old	Age, gender, BMI, environmental lead exposure level, and serum iron, zinc, and calcium	**OR** **Decreased WBC count (<4 × 10^9/L)** 1 (0.905, 1.105)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Werder et al. (2020) Gulf Region United States 2012-2013 Cross-sectional	Gulf Long-Term Follow-up Study n: 214 Non-smoking ≥30 yr old male oil spill response workers and oil spill safety trainees with no history of liver disease or heavy alcohol use	Pb measure in blood using solid-phase micro-extraction with gas chromatography/mass spectrometry Age at measurement: ≥30 Mean: 1.82 µg/dL	IL-6, IL-8, IL-1ß, TNF-α Cytokeratin 18 (CK18 M65 and CK18 M30) Age at Outcome: ≥30	Age, race, alcohol consumption, serum cotinine, BMI, diabetes diagnosis, and education	**pg/mL change (obese participants)** **IL-6** 169.6 (119.8, 219.4) **IL-8** 360.9 (246.2, 475.6) **IL-1β** 76.3 (63.6, 89.0) **TNF-β** 1.1 (-1.5, 3.6)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Zhang et al. (2020) Shantou China Cross-sectional	n: 147 Children 3–7 yr old at two kindergartens (one near an e-waste facility, and the other in a matched reference area)	Blood Blood Pb measured in venous whole blood using GFAAS Age at measurement: 3–7 yr old Median: Reference kindergarten: 2.3 µg/dL; Exposed (e-waste) kindergarten: 3.7 µg/dL	Neutrophils, monocytes, lymphocytes, IL-1ß, IL-6, IL-8, IL-10, and TNF-α Immune cells measured in whole blood using an automated blood cell analyzer; Serum cytokines measured using the ProcartaPlex Human Cytokine Chemokine Panel 1A Age at Outcome: 3–7 yr old	Gender, age, BMI, e-waste contamination w/ in 50 m of residence, residence as workplace, distance of residence from road, family member daily smoking, maternal work associated w/ e-waste, child contact w/ e-waste, washing hands before eating, milk consumption frequency, and ventilation of house	Per natural log increase in erythrocyte Pb **ln(Neutrophils)** 0.20 (0.00, 0.39) **ln(Monocytes)** 0.02 (-0.14, 0.18) **ln(Lymphocytes)** -0.05 (-0.24, 0.16) **ln(IL-1β)** 0.19 (-0.08, 0.45) **ln(IL-6)** 0.33 (0.04, 0.62) **ln(IL-8)** 0.05 (-0.28, 0.37) **ln(IL-10)** 0.08 (-0.29, 0.44) **ln(TNF-α)** -0.18 (-0.44, 0.08)

BLL = blood lead level; BMI = body mass index; CD = cluster of differentiation; CI = confidence interval; CK = cytokeratin; CR1 = complement receptor type 1; e-waste = electronic-waste; ELISA = enzyme-linked immunosorbent assay; GFAAS = graphite furnace atomic absorption spectrometry; HBc = Hepatitis B core; HBsAb = Hepatitis B surface antigen; HBV = Hepatitis B virus; Hib = *Haemophilus influenzae* type B; ICP-MS = inductively coupled plasma mass spectrometry; Ig– = immunoglobulin type; IL = interleukin type; IFN-g = interferon-gamma; ln = natural logarithm; mo = month; MRSA = methicillin-resistant *Staphylococcus aureus*; NHANES = National Health and Nutrition Examination Survey; NR = not reported; OR = odds ratio; Pb = lead; PCR = polymerase chain reaction; RSV = respiratory syncytial virus; S/CO = signal to cut-off; SES = socioeconomic status; SPT = skin prick test; TNF-a = tumor necrosis factor alpha; WBC = white blood cell; yr = year(s).

[a] Effect estimates are standardized to a 1 µg/dL increase in blood Pb level or a 10 µg/g increase in bone Pb level, unless otherwise noted. For studies that report results corresponding to a change in log-transformed Pb biomarkers, effect estimates are assumed to be linear within the 10th to 90th percentile interval of the biomarker and standardized accordingly.

† Studies published since the 2013 Pb ISA.

1

Table 6-5 Animal toxicological studies of delayed-type hypersensitivity responses.

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Fang et al. (2012)	**Rat (**Sprague Dawley**)** Control (vehicle), M, n = 20 300 ppm Pb, M, n = 20	23–25 d to 65–67 d	Dosing solutions were changed twice per wk	4.48 µg/dL for 0 ppm 18.48 µg/dL for 300 ppm - d 65–67	DTH

BLL = blood lead level; d = day; DTH = delayed-type hypersensitivity; M = male; MMR = measles, mumps, and rubella; Pb = lead; ppm = parts per million; wk = week

[a] If applicable, reported values for BLL were converted to ∝g/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-6 Animal toxicological studies of antibody response.

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Yathapu et al. (2020)	**Rat (**Sprague Dawley**)** Control (vehicle) M/F, n = 32 (16/16)	PND 54 – PND 82	Weanling rats (PND 21) were acclimated to the facility for 5 days before being divided into two groups (n = 16) to begin a 28-day long Fe deficiency diet. After 28 days, the rats were exposed to Pb or control diet (n = 16). At this point (PND 82), blood was collected from rats before immunization with TT (n = 8) followed by two boosters administered in 2-wk intervals. Vaccine response was evaluated 2 wk later	2.1 ± 1.0 µg/dL for 0 mg/4 mL/kg, 16.1 ± 5.5 µg/dL for 25 mg/4 mL/kg - PND 82, Control diet 1.9 ± 0.7 µg/dL for 0 mg/4 mL/kg 41.6 ± 10.2 µg/dL for 25 mg/4 mL/kg - PND 82, Iron deficiency diet	Vaccine response, Antigen-specific antibodies

BLL = blood lead level; Fe = iron; M/F = male/female; Pb = lead; PND = postnatal day; TT = tetanus toxoid.

[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-7 Animal toxicological studies of ex vivo white blood cell function.

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Fang et al. (2012)	**Rat (**Sprague Dawley**)** Control (vehicle), M, n = 20 300 ppm Pb, M, n = 20	23–25 d to 65–67 d	Dosing solutions were changed twice per wk.	4.48 µg/dL for 0 ppm, 18.48 µg/dL for 300 ppm –– d 65-67	Treg cell suppression assay

Yathapu et al. (2020)	**Rat (**Sprague Dawley**)** Control (vehicle), M/F, n = 32 (16/16) 500 ppm Pb, M/F, M/F, n = 32 (16/16)	PND 54 – PND 82	Weanling rats (PND 21) were acclimated to the facility for 5 days before being divided into two groups (n = 16) to begin a 28-day long Fe deficiency diet. After 28 days, the rats were exposed to Pb or control diet (n = 16). At this point (PND 82), blood was collected from rats before immunization with TT (n = 8) followed by two boosters administered in 2-wk intervals. Vaccine response was evaluated 2 wk later.	2.1 ± 1.0 µg/dL for 0 mg/4 mL/kg 16.1 ± 5.5 µg/dL for 25 mg/4 mL/kg - PND 82, Control diet 1.9 ± 0.7 µg/dL for 0 mg/4 mL/kg 41.6 ± 10.2 µg/dL for 25 mg/4 mL/kg - PND 82, Iron deficiency diet	Spleen cell proliferation

BLL = blood lead level; d = day; Fe = iron; M/F = male/female; Pb = lead; PND = postnatal day; ppm = parts per million; Treg = regulatory T cells; TT = tetanus toxoid; wk = week.
[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-8 Animal toxicological studies of immune organ pathology.

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL) [a]	Endpoints Examined
Corsetti et al. (2017)	**Mouse (C57BJ)** Control (vehicle), M, n = 8 200 ppm Pb, M, n = 8	30–75 d	Mice were exposed via drinking water for 45 consecutive days. Control animals were exposed to drinking water containing acetic acid (1 mL/L)	<5 µg/dL for 0 ppm 21.6 µg/dL for 200 ppm	Spleen histopathology
Dumková et al. (2017)	**Mouse (ICR)** Control (vehicle), F, n = 10 1.23 × 10^6 particles/cm^3 Pb, F, n = 10	NR	Mice were exposed continuously (24 h/d, 7 d/wk) for 6 wk. Control animals were exposed to the same air as the treated group without the addition of Pb nanoparticles. The investigators pooled animals from two independent experiments, each with five animals per treatment	11 ng/g for 0 × 10^6 particles/cm^3 Pb (1.166 µg/dL) 132 ng/g for 1.23 × 10^6 particles/cm^3 Pb (13.992 µg/dL)	Spleen histopathology
Dumková et al. (2020b)	**Mouse CD-1 (ICR)** Control (vehicle), F, n = 10 (2 wk, 6 wk, 11 wk) 2.23 × 10^6 NPs/cm^3 PbO NP, F, n = 10 (2 wk, 6 wk, 11 wk) 2.23 × 10^6 NPs/cm^3 PbO NP recovery, F, n = 10 (6 wk PbO NP, 5 wk clean air)	NR	Mice (unknown age) were exposed to clean air or PbO NPs 24 hr/d 7 d/wk for 2 wk, 6 wk, or 11 wk. a recovery group was exposed to PbO NPs for 6 wk and then clean air for 5 wk (11 wk total)	<3 ng/g for 0 PbO NPs/cm^3 (<0.3 µg/dL) 104 ng/g for 2.23 × 10^6 NPs/cm^3 - 2 wk (10.4 µg/dL) <3 ng/g for 0 PbO NPs/cm^3 – 6 wk (<0.3 µg/dL) 148 ng/g for 2.23 × 10^6 NPs/cm^3 - 6 wk (14.8 µg/dL) <3 ng/g for 0 PbO NPs/cm^3 -11 wk (<0.3 µg/dL)	Spleen histopathology

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL) [a]	Endpoints Examined
				174 ng/g for 2.23 × 10^6 NPs/cm^3 - 11 wk (17.4 µg/dL) <3 ng/g for 0 PbO NPs/cm^3 (<0.3 µg/dL) 27 ng/g - recovery (6 wk PbO NP, 5 wk clean air) (2.7 µg/dL)	
Dumková et al. (2020a)	**MouseCD-1 (ICR)** Control (vehicle), F, n = 10 68.6 µg/m^3 Pb, F, n = 10	6–8 wk old mice exposed for 3 d, 2 wk, 6 wk, or 11 wk	Mice were exposed to Pb for 3 d, 2 wk, 6 wk, or 11 wk. To assess recovery, a separate group of mice were exposed for 11 wk followed by 5 wk of clean air. Control group was exposed to filtered air	<0.3 ng/g for control at all timepoints (d 3, 2 wk, 6 wk, 11 wk) (<0.3 µg/dL) 31 ng/g for 68.6 µg/m^3 Pb - d 3 (3.1 µg/dL) 40 ng/g for 68.6 µg/m^3 Pb - 2 wk (4.0 µg/dL) 47 ng/g for 68.6 µg/m^3 Pb - 6 wk (4.7 µg/dL) 85 ng/g for 68.6 µg/m^3 Pb - 11 wk (8.5 µg/dL) 10 ng/g for 68.6 µg/m^3 Pb - 6 wk exposure plus 5 wk clean air (1.0 µg/dL)	Spleen histopathology

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL) [a]	Endpoints Examined
Smutná et al. (2022)	**Mouse CD-1 (ICR)** Control (vehicle), F, n = 10 0.956 µg/m^3 Pb, F, n = 10	6–8 wk old mice exposed for 11 wk	Mice were exposed to Pb for 11 wk. Control group was exposed to filtered air	<0.003 ± 0.001 ng/g for control at 11 wk (0.318 ± 0.106 µg/dL) 0.171 ± 0.012 ng/g for 0.956 µg/m^3 Pb - 11 wk (18.126 ± 1.272 µg/dL)	Spleen histopathology

BLL = blood lead level; d = day; F = female; Pb = lead; PbO nanoparticles = lead oxide nanoparticles; ppm = parts per million; wk = week.

[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-9 Animal toxicological studies of immunoglobulin levels.

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Yathapu et al. (2020)	Rat (Sprague Dawley) Control (vehicle) M/F, n = 32 (16/16) 500 ppm Pb, M/F, n = 32 (16/16)	PND 54 – PND 82	Weanling rats (PND 21) were acclimated to the facility for 5 days before being divided into two groups (n = 16) to begin a 28-day long Fe deficiency diet. After 28 days, the rats were exposed to Pb or control diet (n = 16). At this point (PND 82), blood was collected from rats before immunization with TT (n = 8) followed by two boosters administered in 2- wk intervals. Vaccine response was evaluated 2 wk later.	2.1 ± 1.0 ∝g/dL for 0 mg/4 mL/kg – PND 82, Control Diet 16.1 ± 5.5 ∝g/dL for 25 mg/4 mL/kg – PND 82, Control diet 1.9 ± 0.7 ∝g/dL for 0 mg/4 mL/kg – PND 82, Iron deficiency diet 41.6 ± 10.2 ∝g/dL for 25 mg/4 mL/kg – PND 82, Iron deficiency diet	Immunoglobulin levels

BLL = blood lead level; Fe = iron; M/F= male/female; Pb = lead; PND = postnatal day; TT = tetanus toxoid.

[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-10 Animal toxicological studies of immune organ weight.

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Amos-Kroohs et al. (2016)	**Rat (**Sprague Dawley**)** Control (vehicle), M/F, n = 4 (2/2) 1 mg/kg Pb, M/F, n = 16 (8/8) 10 mg/kg Pb, M/F, n = 16 (8/8)	PND 4 – PND 28	Male and female rats were gavaged every other day from PND 4 to PND 10, 18, or 28. Starting on PND 4, ISO offspring were isolated from their dam individually for 4 h. Control animals remained with their dam throughout this period. On PND 11, 19, or 29, subsets within each group were subjected to acute stressor (shallow water stressor for 0, 30, or 60 min) or left undisturbed. Control animals were gavaged with vehicle containing anhydrous sodium acetate (0.01 M)	1.19 µg/dL for 0 mg/kg 2.73 µg/dL for 1 mg/kg 9.15 µg/dL for 10 mg/kg – PND 29 w/o ISO stress 1.31 µg/dL for 0 mg/kg, 4.55 µg/dL for 1 mg/kg 17.1 µg/dL for 10 mg/kg – PND 29 w/ ISO stress	Spleen weight, Thymus weight
Corsetti et al. (2017)	**Mouse (C57BJ)** Control (vehicle), M, n = 8 200 ppm Pb, M, n = 8	d 30–d 75	Mice were exposed via drinking water for 45 consecutive days. Control animals were exposed to drinking water containing acetic acid (1 mL/L)	<5 µg/dL for 0 ppm 21.6 µg/dL for 200 ppm	Spleen weight
Dumková et al. (2017)	**Mouse (ICR)** Control (vehicle), F, n = 10 1.23×10^6 particles/cm^3 Pb, F, n = 10	NR	Mice were exposed continuously (24 h/d, 7 d/wk) for 6 wk. Control animals were exposed to the same air as the treated group without the addition of Pb nanoparticles. The investigators pooled animals from two independent experiments, each with five animals per treatment	11 ng/g for 0×10^6 particles/cm^3 Pb (1.166 µg/dL) 132 ng/g for 1.23×10^6 particles/cm^3 Pb (13.992 µg/dL)	Spleen weight

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Dumková et al. (2020b)	**Mouse CD-1 (ICR)** Control (vehicle), F, n = 10 (2 wk, 6 wk, 11 wk) 2.23 × 10^6 NPs/cm^3 PbO NP, F, n = 10 (2 wk, 6 wk, 11 wk) 2.23 × 10^6 NPs/cm^3 PbO NP recovery, F, n = 10 (6 wk PbO NP, 5 wk clean air)	NR	Mice (unknown age) were exposed to clean air or PbO NPs 24 hr/d 7d/wk for 2 wk, 6 wk, or 11 wk. a recovery group was exposed to PbO NPs for 6 wk and then clean air for 5 wk (11 wk total)	<3 ng/g for 0 PbO NPs/cm^3– 2 wk (<0.3 µg/dL) 104 ng/g for 2.23 × 10^6 NPs/cm^3 – 2 wk (10.4 µg/dL) <3 ng/g for 0 PbO NPs/cm^3 – 6wk (<0.3 µg/dL) 148 ng/g for 2.23 × 10^6 NPs/cm^3 – 6 wk (14.8 µg/dL) <3 ng/g for 0 PbO NPs/cm^3 – 11 wk (<0.3 µg/dL) 174 ng/g for 2.23 × 10^6 NPs/cm^3 – 11 wk (17.4 µg/dL) <3 ng/g for 0 PbO NPs/cm^3 (<0.3 µg/dL) 27 ng/g – recovery (6 wk PbO NP, 5 wk clean air) (2.7 µg/dL)	Spleen weight
Dumková et al. (2020a)	**Mouse CD-1 (ICR)** Control (vehicle), F, n = 10 68.6 µg/m^3 Pb, F, n = 10	6–8 wk old mice exposed for 3 d, 2 wk, 6 wk, or 11 wk	Mice were exposed to Pb for 3 d, 2 wk, 6 wk, or 11 wk. To assess recovery, a separate group of mice were exposed for 11 wk followed by 5 wk of clean air. Control group was exposed to filtered air	<0.3 ng/g for control at all timepoints (d 3, 2 wk, 6 wk, 11 wk) (<0.3 µg/dL) 31 ng/g for 68.6 µg/m^3 Pb – d 3 (3.1 µg/dL) 40 ng/g for 68.6 µg/m^3 Pb – 2 wk (4.0 µg/dL)	Spleen weight

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
				47 ng/g for 68.6 µg/m^3 Pb – 6 wk (4.7 µg/dL) 85 ng/g for 68.6 µg/m^3 Pb – 11 wk (8.5 µg/dL) 10 ng/g for 68.6 µg/m^3 Pb – 6 wk exposure plus 5 wk clean air (1.0 µg/dL)	
Smutná et al. (2022)	**Mouse CD-1 (ICR)** Control (vehicle), F, n = 10 0.956 µg/m^3 Pb, F, n = 10	6–8 wk old mice exposed for 11 wk	Mice were exposed to Pb for 11 wk. Control group was exposed to filtered air	<0.003 ± 0.001 ng/g for control at 11 wk (0.318 ± 0.106 µg/dL) 0.171 ± 0.012 ng/g for 0.956 µg/m^3 Pb - 11 wk (18.126 ± 1.272 µg/dL)	Spleen histopathology
Graham et al. (2011)	**Rat (**Sprague Dawley**)**,	PND 4–PND 28	Dosed every other day. Control animals were gavaged with vehicle containing anhydrous sodium acetate (0.01 M)	0.267 µg/dL for 0 mg/kg, 3.27 µg/dL for 1 mg/kg, 12.5 µg/dL for 10 mg/kg – PND 29	Spleen weight, Thymus weight

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
	Groups: PND 11 Control (vehicle), M/F, n = 192 (96/96) 1 mg/kg Pb, M/F, n = 192 (96/96) 10 mg/kg Pb, M/F, n = 191 (96/95) PND 19 Control (vehicle), M/F, n = 191 (96/95) 1 mg/kg Pb, M/F, n = 191 (96/95) 10 mg/kg Pb, M/F, n = 192 (96/96) PND 29 Control (vehicle), M/F, n = 192 (96/96) 1 mg/kg Pb, M/F, n = 192 (96/96) 10 mg/kg Pb, M/F, n = 192 (96/96)				
Graham et al. (2011)	**Rat (**Sprague Dawley**)**	PND 4–PND 28	Dosed every other day. Control animals were gavaged with vehicle containing anhydrous sodium acetate (0.01 M)	0.267 µg/dL for 0 mg/kg – PND 29 3.27 µg/dL for 1 mg/kg – PND 29 12.5 µg/dL for 10 mg/kg – PND 29	Spleen weight, Thymus weight

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
	Groups: PND 11 Control (vehicle), M/F, n = 192 (96/96) 1 mg/kg Pb, M/F, n = 192 (96/96) 10 mg/kg Pb, M/F, n = 191 (96/95) PND 19 Control (vehicle), M/F, n = 191 (96/95) 1 mg/kg Pb, M/F, n = 191 (96/95) 10 mg/kg Pb, M/F, n = 192 (96/96) PND 29 Control (vehicle), M/F, n = 192 (96/96) 1 mg/kg Pb, M/F, n = 192 (96/96) 10 mg/kg Pb, M/F, n = 192 (96/96)				

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Wildemann et al. (2015)	**Rat (Wistar)** Control (vehicle), M, n = 6 357 µg/kg/d Pb, M, n = 5 1607 µg/kg/d Pb, M, n = 5	NR	Control group provided tap water with 0.2% nitric acid	1.4 ± 1.2 µg/L for 0 µg/kg/d (0.14 µg/dL) 17 ± 7 µg/L for 357 µg/kg/d (1.77 ± 0.7 µg/dL) 86 ± 29 µg/L for 1607 µg/kg/d (0.14 µg/dL for 0 µg/kg/d, 1.77 ± 0.7 µg/dL for 357 µg/kg/d, 8.6 ± 2.9 µg/dL for 1607 µg/kg/d)	Spleen weight

BLL = blood lead level; d = day; M = male; M/F = male/female; F = female; h = hour; ISO = isolation, min = minute; NR = not reported; Pb = lead; PbO NPs = lead oxide nanoparticles; PND = postnatal day; ppm = parts per million; w/o = without; wk = week.

[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-11 Animal toxicological studies of white blood cell counts and differentials (spleen, thymus, lymph node, bone marrow).

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Cai et al. (2018)	**Rat (**Sprague Dawley**)** Control (vehicle), M/F, n = 5 0.2% Pb, M/F, n = 5	8–10 wk to 20–30 wk	Rats were 8-10 wk old when acquired. Whether or not the rats were allowed to acclimate to the facility prior to study initiation was not reported. The number of males and females not reported. Control animals received tap water	20.5 ± 0.68 µg/L for 0% (2.2 ± 6.4 µg/dL) 87.4 ± 9.2 µg/L for 0.2% (9.3 ± 0.98 µg/dL)	Bone marrow cell counts and differentials
Fang et al. (2012)	**Rat (**Sprague Dawley**)** Control (vehicle), M, n = 20 300 ppm Pb, M, n = 20	23–25 d to 65–67 d	Dosing solutions were changed twice per week	4.48 µg/dL for 0 ppm 18.48 µg/dL for 300 ppm – d 65-67	Thymus cell counts and differentials, Spleen cell counts and differentials, Lymph node cell counts and differentials
Yathapu et al. (2020)	**Rat (**Sprague Dawley**)** Control (vehicle), M/F, n = 32 (16/16) 500 ppm Pb, M/F, n = 32 (16/16)	PND 54–PND 82	Weanling rats (PND 21) were acclimated to the facility for 5 days before being divided into two groups (n = 16) to begin a 28-day long Fe deficiency diet. After 28 d, the rats were exposed to Pb or control diet (n = 16). At this point (PND 82), blood was collected from rats before immunization with TT (n = 8) followed by two boosters administered in 2 wk intervals. Vaccine response was evaluated 2 wk later	2.1 ± 1.0 µg/dL for 0 mg/4 mL/kg – PND 82, Control diet 16.1 ± 5.5 µg/dL for 25 mg/4 mL/kg – PND 82, Control diet 1.9 ± 0.7 µg/dL for 0 mg/4 mL/kg – PND 82, Iron deficiency diet 41.6 ± 10.2 µg/dL for 25 mg/4 mL/kg – PND 82, Iron deficiency diet	Spleen cell counts and differentials

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Zhu et al. (2020)	**Mouse (C57BL.6)** Control (vehicle), M/F, n = NR 125 ppm Pb, M/F, n = NR 1250 ppm Pb, M/F, n = NR	7–9 wk	Control animals were exposed to drinking water containing sodium acetate. The investigators specified that an equal number of male and female mice were used in the study, but the number of animals used in some analyses was not an even number. Consequently, it is not possible to determine sex composition of the group and it suggests there may have been unreported attrition	0 µg/dL for 0 ppm 4.7 ± 0.2 µg/dL for 125 ppm 41.3 µg/dL for 1250 ppm	Spleen cell counts and differentials, Bone marrow cell counts and differentials, Lymph node cell counts

BLL = blood lead level; d = day; M/F = male/female; NR = not reported; Pb = lead; PND = postnatal day; ppm = parts per million; wk = week; TT = tetanus toxoid.

[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-12 Animal toxicological studies of white blood cell counts (hematology and subpopulations).

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Andjelkovic et al. (2019)	**Rat (Wistar)** Control (vehicle), M, n = 8 0.2% Pb, M, n = 6	NR	Rats (250 g), age at time of dosing not reported, were exposed to a single dose of 150 mg Pb/kg BW Pb acetate via oral gavage. Control animals were given "water"	24.9 ± 19 µg/kg for 0 mg Pb/kg BW (2.6 ± 2.0 µg/dL) 291.2 ± 139 µg/kg for 150 mg Pb/kg BW (30.9 ± 14.7 µg/dL)	WBC counts, WBC subpopulations
Cai et al. (2018)	**Rat (**Sprague Dawley**)** Control (vehicle), M/F, n = 5 0.2% Pb, M/F, n = 5	8–10 wk to 20–30 wk	Rats were 8–10 wk old when acquired. Whether or not the rats were allowed to acclimate to the facility prior to study initiation was not reported. The number of males and females not reported Control animals received tap water	20.5 ± 0.68 µg/L for 0% (2.2 ± 6.4 µg/dL) 87.4 ± 9.2 µg/L for 0.2% (9.3 ± 0.98 µg/dL)	WBC counts
Corsetti et al. (2017)	**Mouse (C57BJ)** Control (vehicle), M, n = 8 200 ppm Pb, M, n = 8	30–75 d	Mice were exposed via drinking water for 45 consecutive days. Control animals were exposed to drinking water containing acetic acid (1 mL/L)	<5 µg/dL for 0 ppm 21.6 µg/dL for 200 ppm	WBC counts
Zhu et al. (2020)	**Mouse (C57BL.6)** Control (vehicle), M/F, n = NR 125 ppm Pb, M/F, n = NR 1250 ppm Pb, M/F, n = NR	7–9 wk	Control animals were exposed to drinking water containing sodium acetate. The investigators specified that an equal number of male and female mice were used in the study, but the number of animals used in some analyses was not an even number. Consequently, it is not possible to determine sex composition of the group and it suggests there may have been unreported attrition	0 µg/dL for 0 ppm 4.7 ± 0.2 µg/dL for 125 ppm 41.3 µg/dL for 1250 ppm	WBC subpopulations

BW = body weight; d = day; F = female; M = male; M/F = male/female; NR = not reported; Pb = lead; ppm = parts per million; WBC = white blood cell; wk = week.

[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-13 Epidemiologic studies of exposure to Pb and sensitization and allergic response.

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates (EEs) and 95% CIs[a]
†Ashley-Martin et al. (2015) Canada 2008–2011 Cohort	Maternal-Infant Research on Environmental Chemicals Study n: 1256 Pregnant women were recruited at <4 wk gestation. Singleton non-pre-term births	Maternal/Cord Blood Blood Pb was measured in whole blood using ICP-MS; concentrations measured in the first and third trimester were averaged to create an index of exposure throughout pregnancy Age at measurement: First and third trimesters Median: 0.62 µg/dL Maximum: 4.14 µg/dL	IL-33, TSLP, and IgE IL-33, TSLP, and IgE measured in cord blood plasma using a commercial antibody kit and ELISA. Age at Outcome: At birth	Age	**ORs per 10-fold increase in Pb** **Elevated IL-33/TSLP** 0.72 (0.48, 0.95) **Elevated IgE** 0.98 (0.66, 1.3)
Joseph et al. (2005) Southeastern Michigan 1994–1997 Enrollment (Follow-up for 12 mo after Pb screening) Cohort	n: 4,634 Children enrolled in a managed care organization. Enrollment at 4 mo to 3 yr	Blood Blood Pb measured in venous whole blood using GFAAS. Age at measurement: 4 mo to 3 yr Mean: 4.7 µg/dL (SD: 4.0)	Incident Asthma Four or more asthma-medication–dispensing events in 12 mo or met one or more of the following within a 12-mo period: emergency department visit for asthma, hospitalization for asthma, or four or more outpatient visits for asthma with at least two asthma-medication–dispensing events	Sex, birth weight, and average annual income available only at census block level	HRs: **White children, ≥5 vs. <5 µg/dL:** 2.7 (0.9, 8.1) **Black children, ≥10 vs. <5 µg/dL:** 1.3 (0.6, 2.6)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates (EEs) and 95% CIs[a]
†Kim et al. (2019) Seoul South Korea 2007–2011 enrollment (at least 2 yr of follow-up) Cohort	Cohort for Childhood Origin of Asthma and Allergic Disease n: 331 Pregnant women enrolled in third trimester, children followed at least 2 yr	Maternal/Cord Blood Cord blood Pb measured using ICP-MS Age at measurement: At birth Median: 1.3 µg/dL Maximum: 4.3 µg/dL	Atopic Dermatitis and IL-13 IL-13 measured in cord blood; diagnosis of atopic dermatitis by pediatric allergists, and atopic dermatitis scored using a validated measure (SCORAD) Age at Outcome: At birth (IL-13), 6 mo, 12 mo, and 2 yr	Gender and parental history of allergic diseases	**EEs per unit increase in ln(Pb)** **HR Atopic Dermatitis** 0.96 (0.60, 1.53) **ln(SCORAD)** **Atopic Dermatitis Severity** 1.11 (−2.65, 4.87) **IL-13 (pg/ml)** 0.69 (0.11, 1.28)
†[b]Kim et al. (2013) South Korea 2006–2009 enrollment (follow-up with infants 6 mo after birth) Cohort	Mothers' and Children's Environmental Health Study n: 637 Singleton children of mothers enrolled between weeks 12 and 28 of gestation	Maternal/Cord.Blood Cord blood Pb measured using GFAAS Age at measurement: At birth Mean: 1.01 µg/dL	Atopic Dermatitis Age at Outcome: 6 mo	Age, weight, history of atopic disease, maternal education, infant sex, family income, family size, parity, duration of breast feeding, passive smoking during pregnancy, and cord blood cadmium	**OR** **Atopic Dermatitis** 1.05 (0.60, 1.81)
†Kim et al. (2016) South Korea 2010–2011 Cross-Sectional	KNHANES n: 2184 General population; 26–55 yr old	Blood Blood Pb was measured in venous whole blood using GFAAS Age at measurement: 26–55 yr old Median: 2.14 µg/dL 75th: 2.82 µg/dL	IgE Serum total IgE (kU/L) measured by immunoradiometric assay Age at Outcome: 26–55 yr old	Age, sex, urine cotinine, mercury, and cadmium	**% Change in Total IgE (kU/L)** **Sensitization Negative** 3.5% (−1.8%, 9.4%) **Sensitization Positive** 10.4% (3.3%, 17.8%)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates (EEs) and 95% CIs[a]
†Mener et al. (2015) United States 2005–2006 Cross-Sectional	NHANES n: 2,712 children; 4,333 adults General population; children 6–19 yr old, adults ≥20 yr old	Blood Blood Pb was measured in venous whole blood using ICP-MS Age at measurement: ≥6 yr old Serum median: Children: 0.87 µg/dL; Adults: 1.48 µg/dL 75th: Children: 1.31 µg/dL; Adults: 2.34 µg/dL	Immune System Effects Food Allergen-Specific Serum IgE measured using immunoassays Age at Outcome: ≥6 yr old	Age, sex, ethnicity, BMI, exposure to tobacco smoke, asthma, musty smell, presence of cockroaches, and domestic animals living at home, and year home was built	**ORs** **Increased sensitivity to food allergens** **Children** 0.72 (0.48, 0.95) **Adults** 0.98 (0.66, 1.3)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates (EEs) and 95% CIs[a]
†Pesce et al. (2021) Nancy and Poitier France 2003–2006 Enrollment (Follow-up to 8 yr of age) Cohort	EDEN Birth Cohort n: 651 Pregnant women enrolled early in pregnancy, children followed through 8 yr of age	Maternal/Cord Blood Maternal blood Pb measured between 24 and 28 gestational weeks using GFAAS; Cord blood Pb measured at birth using GFAAS Age at measurement: Prenatal Mean: Cord blood: 1.45 µg/dL; Maternal blood: 1.91 µg/dL; Median: Cord blood: 1.2 µg/dL; Maternal blood: 1.7 µg/dL 75th: Cord blood: 1.8 µg/dL; Maternal blood: 2.2 µg/dL	Atopic Diseases Parental questionnaires using validated questions from the International Study on Asthma and Allergies in Childhood Age at Outcome: 4, 8, and 12 mo; 2, 3, 4, and 5 yr; and 8 yr	Sex, Maternity Center, BMI, maternal education, parental smoking, parental history of allergy, maternal smoking in pregnancy, birth weight, gestational age at delivery, type of delivery, manganese, and cadmium	**ORs (Q4, Q1)** **Maternal Blood (>2.2 vs. <1.2):** *Asthma* 1.25 (0.71, 2.2) *Rhinitis* 0.86 (0.51, 1.43) *Eczema* 1.04 (0.73, 1.48) *Food Allergy* 1.02 (0.51, 2.01) **Cord Blood (>1.8 vs. <0.9):** *Asthma* 0.74 (0.41, 1.33) *Rhinitis* 0.64 (0.37, 1.11) *Eczema* 1.35 (0.92, 1.98) *Food Allergy* 0.57 (0.25, 1.34)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates (EEs) and 95% CIs[a]
Pugh Smith and Nriagu (2011) Saginaw, MI Cross-sectional	n: 356 Children residing in low-income and minority households identified by the Statewide Systemic Tracking of Elevated Lead Levels and Remediation (STELLAR) database	Blood Blood Pb measured in venous whole blood	Prevalent Asthma Parental report of asthma diagnosis	Age, sex, income, stories in unit, pet ownership, cockroach problem, persons in home, smoker in home, clutter, highest BLL at address, candles or incense, months of residency, housing tenure, stove type, heating source, air conditioning type, peeling paint, ceiling/wall damage, housing age, water dampness	**OR** **(≥10 vs. <10 µg/dL)** **Asthma** 7.5 (1.3, 42.9)
Rabinowitz et al. (1990) Boston, MA Enrollment: 1979–1981 Follow-up: Unclear Cohort	n: 159 Mother infant pairs recruited from Boston Hospital for Women	Cord Blood Cord blood Pb measured in samples at birth using anodic stripping voltammetry	Prevalent Eczema and Asthma Eczema and asthma prevalence evaluated via parental questionnaire	N/A	**OR** **(≥10, vs. <10 µg/dL)** *Eczema* 1.0 (0.6, 1.6) *Asthma* 1.3 (0.8, 2.0)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates (EEs) and 95% CIs[a]
†Tsuji et al. (2019) Japan 2011–2014 Cross-sectional	Japan Environment and Children's Study n: 14408	Blood, Maternal/Cord Blood Blood Pb measure using ICP-MS Age at measurement: Second/third trimester Mean: 6.44 ng/g Q1: <4.79 ng/g Q4: >7.42 ng/g	Allergen-Specific IgE Allergen-specific serum IgE measured using immunological assays Age at Outcome: First trimester	Age, BMI, allergic diseases, smoking during pregnancy, smoking habits of partner, alcohol consumption during pregnancy, pet ownership, month of blood sample, and geographic region	**ORs (Q4, Q1)** **House Dust Mite Sensitization** 0.91 (0.83, 1.01) **Japanese Cedar Pollen Sensitization** 1.04 (0.94, 1.15) **Animal Dander Sensitization** 0.99 (0.88, 1.12)
†Wei et al. (2019) United States 2005–2006 Cross-Sectional	NHANES n: 4509 General population; all ages	Blood Blood Pb was measured in venous whole blood using ICP-MS Age at measurement: ≥1 yr old Mean: Ages ≥20: 1.75 µg/dL; <20: 1.24 µg/dL **Adults** T1: 0.18–1.09 µg/dL T2: 1.09–1.99 µg/dL T3: 2.00–26.4 µg/dL **Children** T1: 0.18–0.77 µg/dL T2: 0.78–1.36 µg/dL T3: 1.37–55.3 µg/dL	Eczema Self-reported physician's diagnosis of eczema Age at Outcome: ≥1 yr old	Age, gender, ethnicity, education, poverty-income ratio, smoking, alcohol use, sleep, and BMI	**ORs** **Eczema – Adults:** T1: Reference T2: 1.14 (0.75, 1.76) T3: 1.09 (0.62, 1.92) **Eczema – Children:** T1: Reference T2: 0.99 (0.62, 1.58) T3: 0.90 (0.60, 1.35)

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates (EEs) and 95% CIs[a]
†Wells et al. (2014) United States 2005–2006 Cross-Sectional	NHANES n: 1788 General population; children 2–12 yr old	Blood Blood Pb was measured in venous whole blood using ICP-MS Age at measurement: 2–12 yr old Geometric Mean: 1.13 µg/dL	Immune System Effects Serum total IgE), Eosinophils (WBC differential from complete blood counts), Asthma (parental/guardian reported), Atopy (at least one specific IgE > 0.35 kU/L), Allergies (parental/guardian reported) Age at Outcome: 2–12 yr old	Season, age, sex, race/ethnicity, parental education, presence of smokers in the home, prenatal smoke exposure, BMI, presence of cockroaches in the home, and avoidance/removal of pets	**ORs** **Asthma** 1.01 (0.76, 1.35) **Atopy** 1.05 (0.93, 1.18) **% Increase** **Total IgE (kU/L)** 10.3% (3.5%, 17.5%) **Percent Eosinophils** 4.6% (2.4%, 6.8%)

BLL = blood lead level; BMI = body mass index; Cis = confidence intervals; EDEN = Effect of Diet and Exercise on Immunotherapy and the Microbiome; ELISA = enzyme-linked immunosorbent assay; EEs = effects estimates; GFAAS = graphite furnace atomic absorption spectrometry; HR = hazard ratio; ICP-MS = inductively coupled plasma mass spectrometry; Ig- = immunoglobulin type; IL- = interleukin type; KNHANES = Korean National Health and Nutrition Examination Survey; ln = natural logarithm; mo = month; N/A = not applicable; NHANES = National Health and Nutrition Examination Survey; NR = not reported; OR = odds ratio; Pb = lead; Q = quartile; SCORAD = scoring atopic dermatitis; SD = standard deviation; SES = socioeconomic status; T = tertile; TSLP = thymic stromal lymphopoietin; vs. = versus; WBC = white blood cell; wk = week; yr = year.

[a] Effect estimates are standardized to a 1 µg/dL increase in blood Pb level or a 10 µg/g increase in bone Pb level, unless otherwise noted. For studies that report results corresponding to a change in log-transformed Pb biomarkers, effect estimates are assumed to be linear within the 10th to 90th percentile interval of the biomarker and standardized accordingly.

†Studies published since the 2013 Pb ISA.

1

Table 6-14 Animal toxicological studies of immediate-type hypersensitivity.

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Cai et al. (2018)	**Rat (**Sprague Dawley**)** Control (vehicle), M/F, n = 5 0.2% Pb, M/F, n = 5	8–10 wk to 20–30 wk	Rats were 8–10 wk old when acquired. Whether or not the rats were allowed to acclimate to the facility prior to study initiation was not reported. The number of males and females not reported. Control animals received tap water	20.5 ± 0.68 µg/L for 0% (2.2 ± 6.4 µg/dL) 87.4 ± 9.2 µg/L for 0.2% (9.3 ± 0.98 µg/dL)	Blood cytokine levels
Fang et al. (2012)	**Rat (**Sprague Dawley**)** Control (vehicle), M, n = 20 300 ppm Pb, M, n = 20	23–25 d to 65–67 d	Dosing solutions were changed twice per week	4.48 µg/dL for 0 ppm 18.48 µg/dL for 300 ppm – d 65–67	Blood cytokine levels

BLL = blood lead level; d = days; M = male; M/F = male/female; ppm = parts per million; wk = weeks.

[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

Table 6-15 Epidemiologic studies of exposure to Pb and autoimmunity and autoimmune disease.

Reference and Study Design	Study Population	Exposure Assessment	Outcome	Confounders	Effect Estimates and 95% CIs[a]
†Joo et al. (2019) South Korea 2008-2013 Cross-sectional	KNHANES n: 32215 General population	Blood Blood Pb was measured in venous whole blood using GFAAS Age at measurement: All ages Mean: Rheumatoid Arthritis: 2.38 µg/dL; Control: 2.44 µg/dL	Rheumatoid Arthritis Self-reported physician diagnosis of rheumatoid arthritis Age at Outcome: All ages	Age, sex, SES, and smoking status	**Rheumatoid Arthritis (OR)** 1.01 (0.89, 1.14)
†Kamycheva et al. (2017) United States 2009–2012 Cross-sectional	NHANES n: 3,643 children and 11,040 adults General population, ≥6 yr old	Blood Blood Pb was measured in venous whole blood using ICP-MS Age at measurement: ≥6 yr Mean: Non-Hispanic White: 1.39 µg/dL; other race/ethnicity: 1.47 µg/dL	Celiac Disease Seropositivity Serum tTG-IgA analyzed with an ELISA Age at Outcome: ≥6 yr	Family income to poverty ratio and race/ethnicity	**Celiac Disease** Mean difference in BLL by CD seropositivity status *Adults* −0.17 µg/dL (−0.54, 0.20) *Children* −0.14 µg/dL (−0.27, −0.02)

BLL = blood lead level; CD = cluster of differentiation; Cis = confidence intervals; ELISA = enzyme-linked immunosorbent assay; Ig- = immunoglobulin type; GFAAS = graphite furnace atomic absorption spectrometry; ICP-MS = inductively coupled plasma mass spectrometry; KNHANES = Korean National Health and Nutrition Examination Survey; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; Pb = lead; SES = socioeconomic status; tTG-IgA = tissue transglutaminase immunoglobulin A; yr = years

[a] Effect estimates are standardized to a 1 µg/dL increase in blood Pb level or a 10 µg/g increase in bone Pb level, unless otherwise noted. For studies that report results corresponding to a change in log-transformed Pb biomarkers, effect estimates are assumed to be linear within the 10th to 90th percentile interval of the biomarker and standardized accordingly.

† Studies published since the 2013 Pb ISA.

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Table 6-16 Animal toxicological studies of autoimmunity and autoimmune disease.

Study	Species (Stock/Strain), n, Sex	Timing of Exposure	Exposure Details (Concentration, Duration)	BLL as Reported (µg/dL)[a]	Endpoints Examined
Fang et al. (2012)	**Rat (**Sprague Dawley**)** Control (vehicle), M, n = 20 300 ppm Pb, M, n = 20	23–25 d to 65–67 d	Dosing solutions were changed twice per week	4.48 µg/dL for 0 ppm 18.48 µg/dL for 300 ppm – d 65–67	Treg cell suppression assay

BLL = blood lead level; d = day; M = male; ppm = parts per million; Treg = regulatory T cell.

[a]If applicable, reported values for BLL were converted to mg/dL using WebPlot Digitizer (https://apps.automeris.io/wpd/) and are shown in parenthesis.

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6.9 References

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