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Review

Cross-species coherence in effects and modes of action in support of causality determinations in the U.S. Environmental Protection Agency's Integrated Science Assessment for Lead

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ABSTRACT

The peer-reviewed literature on the health and ecological effects of lead (Pb) indicates common effects and underlying modes of action across multiple organisms for several endpoints. Based on such observations, the United States (U.S.) Environmental Protection Agency (EPA) applied a cross-species approach in the 2013 Integrated Science Assessment (ISA) for Lead for evaluating the causality of relationships between Pb exposure and specific endpoints that are shared by humans, laboratory animals, and ecological receptors (i.e., hematological effects, reproductive and developmental effects, and nervous system effects). Other effects of Pb (i.e., cardiovascular, renal, and inflammatory responses) are less commonly assessed in aquatic and terrestrial wildlife limiting the application of cross-species comparisons. Determinations of causality in ISAs are guided by a framework for classifying the weight of evidence across scientific disciplines and across related effects by considering aspects such as biological plausibility and coherence. As illustrated for effects of Pb where evidence across species exists, the integration of coherent effects and common underlying modes of action can serve as a means to substantiate conclusions regarding the causal nature of the health and ecological effects of environmental toxicants.

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Abbreviations: δ-ALA, delta aminolevulinic acid; ALAD, aminolevulinic acid dehydratase; AOP, adverse outcome pathway; BW, body weight; CASAC, Clean Air Scientific Advisory Committee; DNA, deoxyribonucleic acid; EPA, Environmental Protection Agency; FSH, follicle stimulating hormone; GABA, gamma aminobutyric acid; GnRH, gonadotropin releasing hormone; Hb, hemoglobin; HERO, Health and Environmental Research Online; HPG, hypothalamic–pituitary–gonadal; IGF-1, insulin-like growth factor; ISA, Integrated Science Assessment; LH, luteinizing hormone; MOA(s), mode(s) of action; NAAQS, National Ambient Air Quality Standards; Pb, lead; ROS, reactive oxygen species; U.S., United States; ZPP, zinc–protoporphyrin production.

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1. Introduction

There is increasing recognition in the scientific community of the potential value of integrating scientific information across human and ecological receptors to improve characterization of the risk posed by environmental exposures (Munns et al., 2003; U.S. EPA, 2005a; Vermeire et al., 2007). A cross-species and transdisciplinary approach was proposed for characterization of the effects of Pb to humans and wildlife (Pokras et al., 2009; Pokras and Kneeland, 2008). Consistent with these developments, the United States (U.S.) Environmental Protection Agency (EPA) used a novel cross-disciplinary approach, drawing upon the coherence of the effects of Pb across human and animal species, to form causal determinations for relationships of Pb exposure with health and ecological effects. These causal determinations are described in the recent Integrated Science Assessment (ISA) for Pb (U.S. EPA, 2013a), which is the comprehensive review, synthesis, and evaluation of the most policy-relevant science and serves as the scientific foundation for the review of the National Ambient Air Quality Standards (NAAQS) for Pb in the U.S (U.S. EPA, 2013a). Pb is one of six criteria pollutants for which the U.S. EPA establishes primary and secondary standards for the level of pollutant in the air. The primary NAAQS are established to provide an adequate margin of safety that is requisite to protect public health from air pollution. Secondary standards for Pb provide public welfare protection, including damage to animals, wildlife, soils, crops, and vegetation. The Clean Air Act requires periodic review of the scientific evidence upon which the standards are based and the adequacy of the standards themselves.

In order to determine the causal nature of air pollution-related health or ecological effects, ISAs apply a well-defined framework to integrate scientific evidence and evaluate the overall weight of evidence. This framework, described in detail in the Preamble to the ISA, includes several factors to guide the evaluation of the evidence across all disciplines: consistency, coherence, strength, biological plausibility, temporality, specificity, analogy, and experimental evidence (U.S. EPA, 2013a). These various aspects of the available scientific information, based on the criteria first articulated by Sir Austin Bradford Hill (Hill, 1965), have been widely applied for hazard identification when evaluating human health evidence for the purpose of judging causality (IOM, 2008; IARC, 2006; U.S. EPA, 2005b; CDC, 2004). Although, originally developed for interpretation of epidemiologic results, the Hill aspects have been modified for use with a broader array of data, i.e., epidemiologic, controlled human exposure, ecological, and animal toxicological studies as well as in vitro data (U.S. EPA, 2013a).

Causal inference can be strengthened by the integration of evidence across disciplines. A weak inference from one line of evidence can be addressed by other lines of evidence and their coherence across disciplines can add support to a cause–effect interpretation of the association. For example, for any effect identified in one scientific discipline, other lines of evidence that elucidate the sequence of key events that lead to that effect can add to the weight of evidence by providing biological plausibility for effects. Where effects of Pb observed in humans can support conclusions regarding effects in wildlife and vice-versa (Pokras et al., 2009), consideration of common underlying mechanisms of Pb toxicity across species can provide an additional basis for integration. While human health and ecological effects are typically assessed through the consideration of distinct discipline-specific bodies of literature, both human and ecological toxicology have recently moved toward characterization of underlying pathways conserved across taxa (Perkins et al., 2013; Ankley et al., 2010). Both the mode of action (MOA) approach and the adverse outcome pathway (AOP) approach are based on shared molecular pathways and key initiating events (Meek et al., 2014). An AOP examines the linkages between molecular initiating events and adverse outcomes at higher levels of biological organization that are relevant to risk assessment (Ankley et al., 2010). MOA is defined as a series of causally linked biochemical or biological key events that are empirically observable, measurable, reproducible and necessary steps of a MOA leading to an effect. The MOA approach typically uses a chemical-specific focus to characterize key events, while the AOP approach uses an endpoint specific focus for the same purpose (Ankley et al., 2010; Meek et al., 2014). Although the MOA construct has historically been used in human toxicology and the AOP in ecological contexts, both facilitate characterizing the effects of toxicants on humans and wildlife. Both approaches support the identification of key events that are conserved across taxa and may in turn inform the other for a range chemicals or outcomes.

As noted by Munns et al. (2003), joint examination of health and ecological effects of environmental stressors in risk-based decision-making is supported by shared exposure pathways and commonalities in biological responses across species, including those characterizing potential underlying mechanisms. In the case of Pb, human and ecological receptors share pathways of exposure due to the non-specificity of multiple sources, including current

emissions from industrial operations and historical emissions from industry as well as from combustion of leaded gasoline and abrasion of leaded paint prior to their phase-out. In addition, Pb is a persistent metal that, once emitted, may continue to cycle through air, soil, and water media, increasing the potential for both human and ecosystem exposures (Fig. 1) (U.S. EPA, 2013a).

The objective of this paper is not to describe the rationale for each of the causal determinations formed in the 2013 ISA for Pb but to describe the novel cross-species approach applied by the U.S. EPA in the 2013 ISA for Pb as an example of how scientific information can be integrated across humans and other biota to support hazard identification (U.S. EPA, 2013a). Thus, the focus is a subset of effects evaluated in the 2013 ISA for Pb for which evidence related to Pb exposure in humans and animal toxicology could be applied to ecological receptors and vice-versa. Specifically, this paper describes (1) coherence of the effects of Pb across multiple scientific disciplines (i.e., epidemiology, toxicology, ecotoxicology) for humans, laboratory organisms, and wildlife; (2) where evidence was available, potential underlying key events that inform a common MOA(s) for Pb across multiple disciplines and species; (3) the use of this evidence in the context of the ISA framework to support causal determinations in the 2013 ISA for Pb. The aspects of coherence and biological plausibility substantiated conclusions regarding causality of relationships with Pb exposure and specific health and ecological effects. Although the effects of Pb are not limited to these endpoints, the bodies of evidence on hematological, reproductive and developmental, and nervous system endpoints were best suited to the application of this approach.

2. Methods

To describe how a cross-species approach can be applied in the characterization of the health and ecological effects of environmental toxicants, we used examples from information included in the 2013 ISA for Pb (U.S. EPA, 2013a). The 2013 ISA for Pb builds upon the conclusions of previous Pb assessments conducted by the EPA (U.S. EPA, 2006a, 1986, 1977) and provides a comprehensive review of the peer-reviewed literature published through September 2011, with the bulk of the studies published from 2006 through 2011. Key studies from previous assessments were included if they represented the definitive work in a particular subject area. The process for developing the 2013 ISA for Pb is described in detail in the Preamble to the ISA (U.S. EPA, 2013a). Briefly, studies evaluating health and ecological effects of Pb were identified in both PubMed and Web of Science through a multipronged literature search strategy including a broad pollutant-based search and various targeted searches that focused on specific health or ecological endpoints. The targeted techniques included searches on specific topics, independent review of tables of contents for journals in which relevant papers may be published, identification of relevant literature by expert scientists, review of citations in previous assessments, and recommendation of journal articles by the public and the Clean Air Scientific Advisory Committee (CASAC) during the external review process. These more targeted searches were based on prior knowledge of the key endpoints related to Pb exposure (i.e., nervous system effects, cardiovascular effects, reproductive and developmental effects, and growth) as well as emerging studies in the air pollution and heavy metal toxicity literature.

References identified through the multipronged search strategy were screened by title and abstract by authors of the ISA. Non-English language papers and conference abstracts were excluded. Those references that were determined to be potentially relevant after reading the title were "considered" for inclusion in the ISA and were added to the Health and Environmental Research Online (HERO) database developed by EPA (available to the public at: www.hero.epa.gov). Only those studies or reports that had undergone scientific peer review and had been published or accepted for publication were considered for inclusion.

Emphasis was placed on epidemiological, toxicological, and ecological studies that examine effects in the range of Pb exposures or doses that are defined in the ISA to be relevant to the review of the NAAQS (i.e., generally within one to two orders of magnitude of measured concentrations) (U.S. EPA, 2013a). The majority of experimental animal and epidemiologic studies included in the ISA reported blood Pb levels below 30 μg/dL. Ecological effects observed at or near ambient Pb concentrations in soil, sediment and water were emphasized where available. Ecological effect studies where concentrations of Pb were measured in biota and/or media were weighted more heavily in consideration of the evidence than studies that did not quantify Pb (reported nominal concentrations only). Studies in terrestrial and aquatic organisms that only reported nominal concentrations were included if they provided information on underlying MOAs or if the observations were also reported in additional studies where Pb was quantified. For both health and ecological effects, studies conducted at higher blood Pb or exposure concentrations were considered if they

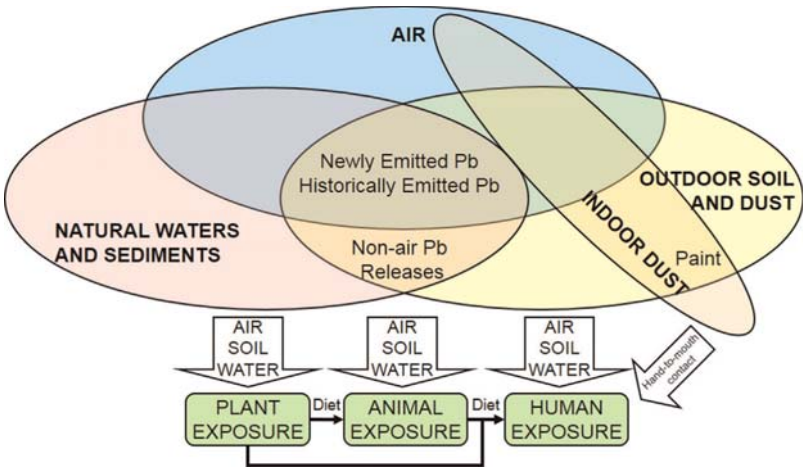

Fig. 1. Conceptual model of joint multimedia Pb exposures among plants, animals, and humans. The Venn diagram depicts how Pb can cycle through multiple environmental media prior to exposure of humans, plants, and/or animals. The "air/soil/water" arrows illustrate Pb exposures directly to the plants, animals, or humans. The flow of arrows from plants to animals to humans represent dietary exposures.

informed the evaluation of kinetics or key events in a MOA. After selecting studies for inclusion, the individual study quality was evaluated by considering the design, methods, and documentation of each study, but not whether the results were positive, negative, or null. This systematic approach to evaluating the literature has gone through extensive peer review and has been supported by various CASAC panels over the course of multiple ISA reviews (Frey, 2012, 2013; U.S. EPA, 2010, 2013b; Frey and Samet, 2011).

Following study quality evaluation, evidence was evaluated across disciplines to develop causal determinations for an array of health and ecological effects. This included the evaluation of strengths and weaknesses in the overall collection of studies across disciplines and confidence in the overall body of evidence, considering the Bradford Hill aspects of consistency, coherence, strength, biological plausibility, temporality, specificity, analogy, and experimental evidence. Separate causal determinations were made for health and ecological effects to inform the primary and secondary NAAQS reviews, respectively, and were based on a five-level hierarchy that classifies the weight of evidence for causation (Table 1). In the case of a causal relationship, the available evidence is sufficient to rule out chance, confounding, and other biases with reasonable confidence. Such evidence may come from a single scientific discipline that has accounted for plausible alternative explanations or from a combination of disciplines that together account for alternative explanations. As uncertainty increases due to limits in the scope and/or the consistency of evidence, the evidence is classified as likely to be a causal, suggestive of a causal, and inadequate to infer a causal relationship. For the evidence determined as not likely to be a causal relationship, studies consistently show no effect. This paper describes the coherence of evidence among species and biological plausibility for health and ecological effects of Pb, focusing on findings that were the basis for determinations of a causal or a likely to be causal relationship in the 2013 ISA for Pb (U.S. EPA, 2013a) (Table 2).

3. Coherence of mode of action and effects

The major key events in the MOAs for Pb effects are outlined below starting with an overview of initial events and followed by information on how they may interact in the development of effects from Pb exposure. The key event underlying effects of Pb in humans and other organisms is alteration of cellular ion status (including disruption of divalent cations, altered ion transport mechanisms, and perturbed protein function through displacement of metal cofactors) (Fig. 2). An example of Pb-induced alteration of ion status is the disruption of calcium (Ca^{2+}) homeostasis. Ca^{2+} is critically important as a cell signal carrier and regulates essential cellular functions (Carafoli, 2005). Exposure to Pb alters the intracellular concentrations of Ca^{2+} in multiple cell types, including bone, brain, red and white blood cells, and gill epithelium (Quintanar-Escorza et al., 2010; Li et al., 2008; Rogers et al., 2005; Ferguson et al., 2000; Schanne et al., 1997). This alteration in intracellular Ca^{2+} concentrations is likely the result of disruptions in ion transport mechanisms, particularly the inhibition of transport proteins such as Na^{+}/K^{+}ATPase and

Table 1
Weight of evidence for causal determination in the Integrated Science Assessments[a].

	Health effects	Ecological and welfare effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in health effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. For example: (a) controlled human exposure studies that demonstrate consistent effects; or (b) observational studies that cannot be explained by plausible alternatives or are supported by other lines of evidence (e.g., animal studies or mode of action information). Evidence includes replicated and consistent high-quality studies by multiple investigators.	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. Controlled exposure studies (laboratory or small- to medium-scale field studies) provide the strongest evidence for causality, but the scope of inference may be limited. Generally, determination is based on multiple studies conducted by multiple research groups, and evidence that is considered sufficient to infer a causal relationship is usually obtained from the joint consideration of many lines of evidence that reinforce each other.
Likely to be a causal relationship	Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures, but important uncertainties remain. That is, the pollutant has been shown to result in health effects in studies in which chance and bias can be ruled out with reasonable confidence but potential issues remain. For example: (a) observational studies show an association, but copollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode of action information) are limited or inconsistent; or (b) animal toxicological evidence from multiple studies from different laboratories that demonstrate effects, but limited or no human data are available. Evidence generally includes replicated and high-quality studies by multiple investigators.	Evidence is sufficient to conclude that there is a likely causal association with relevant pollutant exposures. That is, an association has been observed between the pollutant and the outcome in studies in which chance, bias and confounding are minimized, but uncertainties remain. For example, field studies show a relationship, but suspected interacting factors cannot be controlled, and other lines of evidence are limited or inconsistent. Generally, determination is based on multiple studies in multiple research groups.
Suggestive of a causal relationship	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but is limited. For example, (a) at least one high-quality epidemiologic study shows an association with a given health outcome but the results of other studies are inconsistent; or (b) a well-conducted toxicological study, such as those conducted in the National Toxicology Program (NTP), shows effects in animal species.	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but chance, bias and confounding cannot be ruled out. For example, at least one high-quality study shows an effect, but the results of other studies are inconsistent.
Inadequate to infer a causal relationship	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency or statistical power to permit a conclusion regarding the presence or absence of an effect.	The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of an effect.
Not likely to be a causal relationship	Evidence is suggestive of no causal relationship with relevant pollutant exposures. Several adequate studies, examining relationships with relevant exposures, are consistent in failing to show an effect at any level of exposure.	Several adequate studies, examining relationships with relevant exposures, are consistent in failing to show an effect at any level of exposure.

[a] Adapted from the ISA for Pb (U.S. EPA, 2013a)

Table 2
Summary of causal determinations in the ISA for Lead for health and ecological effects.

Outcome/effect	Health effects Casual determination[a]	Ecological effects Casual determination[a]	Common key events in mode of action
Hematological effects[b]	Causal relationship: red blood cell function and survival, altered heme synthesis	Causal relationship: ALAD activity in terrestrial and freshwater vertebrates Likely causal relationship: ALAD activity in freshwater invertebrates	Altered ion status
Reproductive and developmental effects[c]	Causal relationship: development and male reproductive function	Causal relationship: invertebrates and vertebrates	Endocrine disruption leading to altered hormone homeostasis
Nervous system effects[d]	Causal relationship: cognition, attention, impulsivity and hyperactivity in children Likely causal relationship: conduct disorders, internalizing behaviors, motor function	Likely causal relationship: neurobehavioral effects in terrestrial and freshwater invertebrates and vertebrates	Oxidative stress, altered ion status leading to increased blood brain barrier permeability, changes in brain neuroanatomy, changes in neurotransmitter function
Renal effects	Likely causal relationship: reduced kidney function	N/A[e]	N/A[e]
Immune effects	Likely causal relationship: atopic and inflammatory conditions, decreased host resistance	N/A[e]	N/A[e]
Cardiovascular effects	Causal relationship: hypertension and coronary heart disease	N/A[e]	N/A[e]
Cancer	Likely causal relationship	N/A[e]	N/A[e]
Mortality	N/A[e] (the strongest evidence of Pb-induced mortality in humans was observed for cardiovascular disease related mortality and this evidence was considered in determining the causal relationship between Pb exposure and coronary heart disease).	Causal relationship: survival of terrestrial invertebrates and freshwater invertebrates and vertebrates Likely causal relationship: terrestrial vertebrates	N/A[e]
Growth	N/A[e] (there is mixed evidence from toxicological and epidemiologic studies of Pb effects on postnatal growth, which was considered in determining the causal association between Pb exposure and developmental effects).	Causal relationship: freshwater invertebrates Likely causal relationship: terrestrial invertebrates	N/A[e]
Physiological stress	Was not considered as an outcome or effect but rather an upstream event in the modes of action of Pb.	Likely causal relationship: terrestrial and freshwater invertebrates and vertebrates	In human health, oxidative stress was considered as an upstream event in the modes of action of Pb, leading downstream to various outcomes or effects. Ecological literature commonly uses oxidative stress as a proxy indicator of overall fitness, and thus treats it as an effect.
Community and ecosystem level effects	N/A[e]	Likely causal relationship: terrestrial and freshwater ecosystems	N/A[e]

[a] Causal determinations were made within approximately 1–2 orders of magnitude of current levels of Pb in the environment.
[b] The ecological evidence considered for the causal determination included ALAD activity, blood cell count, and altered serum profiles.
[c] For health effects the strongest evidence was for delayed onset of puberty and detrimental effects on sperm. In the ecological literature, a wide range of endpoints, including embryonic development, multigenerational studies, delayed metamorphosis, and altered steroid profiles, was considered.
[d] In ecological receptors, the causal determination was developed considering neurobehavioral effects that can be observed in toxicological studies of animal models and studies of ecological effects in vertebrates and invertebrates.
[e] N/A, not applicable, outcomes and effects were not directly comparable for the health and ecological evidence or there was insufficient data available across taxa.

voltage-sensitive Ca^{2+} channels (Baranowska-Bosiacka et al., 2011; Rogers et al., 2005; Rogers and Wood, 2004; Audesirk and Audesirk, 1991, 1993). Pb interferes with these proteins through direct competition with native metal cofactors or through disruption of proteins important in Ca^{2+}-dependent cell signaling, such as protein kinase C or calmodulin (Hwang et al., 2002; Kern et al., 2000; Long et al., 1994; Habermann et al., 1983). Pb can also displace other divalent metals such as zinc and magnesium, thus disrupting neurotransmitter function, inhibiting heme synthesis, and decreasing cellular energy production through perturbed mitochondrial function. Pb is able to displace metal ions due to its flexible coordination number (i.e., the number of neighboring atoms in the 3-dimensional protein structure) and its ability to bind multiple ligands. Displacement of native metal ion cofactors by Pb causes abnormal conformational changes in the protein structure and ultimately results in altered protein functions.

A second key event in the MOAs of Pb is the development of oxidative stress, due to the antagonism of normal metal ion functions. The origin of oxidative stress produced after Pb exposure is likely a multipathway process. A major portion of Pb-induced oxidative stress results from Pb binding to and subsequently inhibiting the function of delta aminolevulinic acid dehydratase (ALAD) due to competition of Pb ions with native Zn ions, leading to the accumulation of delta aminolevulinic acid (δ-ALA) in blood and urine. As δ-ALA accumulates, it undergoes tautomerization and autoxidation, resulting in the generation of reactive oxygen species (ROS) (Hermes-Lima et al., 1991; Monteiro et al., 1986, 1989, 1991). Other important sources of Pb-induced oxidative stress are membrane and lipid peroxidation, NAD(P)H oxidation, and antioxidant enzyme depletion.

Both alteration of cellular ion status and oxidative stress can lead to misregulated inflammation, endocrine disruption, cell

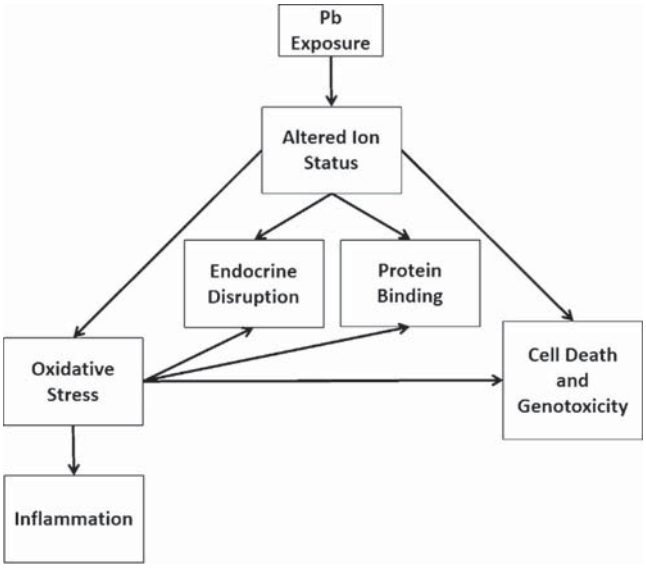

Fig. 2. Schematic representation of the relationships between various key events in the MOAs by which Pb exposure exerts its health effects.

death, protein binding, and genotoxicity (Fig. 2). These are key events in the MOAs for immune, nervous system, reproductive, and developmental effects as well as cancer. Altered ion status and oxidative stress have been linked to neuronal cell damage, impaired synaptic plasticity, and neurotransmitter changes, which are other downstream key events leading to nervous system effects. In the ISA's evaluation of ecological effects of Pb, oxidative stress was considered to be an early key event in a MOA but also a downstream effect, based on its use in the ecological literature as a proxy indicator of overall fitness of an organism (U.S. EPA, 2013a) (Table 2). Evidence characterizing the key events in the MOAs of Pb as well as the coherence in MOAs and their downstream hematological, reproductive, developmental, and nervous system effects across species are described in the sections that follow.

3.1. Hematological effects

Effects of Pb on heme synthesis are well recognized with a large body of evidence from across taxa available. The 1978 NAAQS for Pb (U.S. EPA, 1978) was set to protect against impaired heme synthesis in children. More recent NAAQS reviews have supported the conclusion that Pb exposure results in altered heme synthesis in multiple species (U.S. EPA, 2006a, 2013a). In the 2013 ISA for Pb, the relationship between Pb exposure and altered heme synthesis was determined to be causal for human health based on evidence of decreased ALAD activity in experimental rodent models and supporting evidence in humans (U.S. EPA, 2013a). As ALAD is the key step in the heme biosynthetic pathway, evidence of decreased ALAD activity is a strong surrogate for impaired heme synthesis. For ecological receptors, decreased ALAD activity is a biomarker of Pb exposure and was used as an endpoint in causal determinations. ALAD activity was determined to have causal relationships with Pb in both terrestrial and freshwater vertebrates and a likely to be a causal relationship in freshwater invertebrates. There were fewer studies in freshwater invertebrates available for evaluation. Concordance among humans, laboratory animals, and terrestrial and aquatic organisms provided evidence for consistency of the observed associations and coherence across species for Pb-induced decreased ALAD activity and impaired heme synthesis in the 2013 Pb ISA (Table 3). For some effects (impaired hematopoiesis, increased eryptosis, effects on red blood cell membrane proteins, and effects on red blood cell energy metabolism enzymes) there is less evidence available and determinations of coherence across taxa was not possible.

3.1.1. Pb effects on heme synthesis

Inhibition of ALAD activity is a widely recognized response in humans, laboratory animals and in biota in terrestrial and aquatic environments where Pb is present. ALAD, the second enzyme in the heme biosynthetic pathway, is one of the most researched targets for the molecular toxicity of Pb, and effects on ALAD activity represent a coherent MOA across taxa. Pb-induced alterations in heme synthesis, evidenced by concentration-dependent decreases in ALAD activity have been observed in both adults and children at blood Pb levels relevant to current U.S. air-related exposures (Wang et al., 2010; Ahamed et al., 2005, 2006, 2007). Inhibition of ALAD in adult worker populations has been observed in other studies, but generally at much higher blood Pb levels (Table 3) (Quintanar-Escorza et al., 2007,b; Patil et al., 2006a,b). These human studies demonstrating Pb-induced decrements in ALAD activity are cross-sectional in design and thus there is some degree of uncertainty regarding the direction of effects and the magnitude, frequency, and duration of Pb exposure that contributed to the observed effects. Additionally, while most studies did not control for potential confounding, the findings of those studies are broadly similar to those that controlled for age, sex, smoking status, and alcohol use. The findings of these human studies are supported by a small but consistent body of studies in adult animals that similarly observe decreased ALAD and ferrochelatase (another enzyme in the heme biosynthetic pathway) activities (Table 3) at blood Pb levels relevant to current U.S. air-related exposures (Gautam and Flora, 2010; Rendón-Ramirez et al., 2007; Lee et al., 2005). In ecological studies, decreased ALAD activity is reported across a wide range of taxa exposed to Pb including bacteria, fish, amphibians, birds and mammals (Table 3) (U.S. EPA, 1977, 1986, 2006a, 2013a; Eisler, 2000).

3.1.2. MOA for hematological effects

Causal relationships between Pb exposure and alterations in heme synthesis are supported by evidence describing key events in the MOA for the inhibition of ALAD. Pb is able to bind competitively to the active site of multiple enzymes due to its flexible coordination number and its ability to bind multiple ligands. When Pb displaces a native metal cofactor, the bound enzyme undergoes abnormal conformational changes in the protein structure that result in altered functions. Specifically regarding ALAD, Pb is able to bind competitively to one of ALAD's four Zn-binding sites and decrease the enzyme's activity in human erythrocytes (Simons, 1995). This decrease in ALAD activity impairs the production of heme and can result in decreased hemoglobin levels (see Table 3), ultimately leading to anemia. The crystal structure and amino acid sequence of ALAD is conserved across taxa (Jaffe and Stith, 2007); therefore, evidence for the competition of binding the ALAD Zn-binding sites in humans lends biological plausibility to the observation of Pb-induced decreases in ALAD activity in aquatic and terrestrial species. Additional evidence for key events in the MOA for ALAD includes a recent publication showing epigenetic modification of the ALAD promoter and subsequent suppressed ALAD transcription in a cohort of battery workers with occupational Pb exposure (Li et al., 2011).

3.1.3. The cross-species approach: conclusions for hematological effects of Pb

In summary, coherence across species and disciplines and biological plausibility for a common MOA was demonstrated for

Table 3
Summary of studies included in the 2013 ISA for Lead that provide mode of action (biological plausibility) and cross-species (coherence) evidence for the causal determination for altered heme synthesis.

Description of key evidence with corresponding causal aspect(s) (biological plausibility and/or coherence)	Key references[a]	Pb exposure or blood Pb levels associated with effects
Pb binds heme synthesis enzymes (ALAD; ferrochelatase), displacing metal ions and disrupting the biosynthetic pathway (biological plausibility)		
Pb competitively inhibits the binding of zinc ions necessary for ALAD activity	Simons (1995)	ALAD enzyme inhibition: 10^{-6} μM Pb
Pb inhibits the incorporation of iron into protoporphyrin IX by ferrochelatase, resulting in zinc–protoporphyrin production (ZPP)	Taketani et al. (1985) Mohammad et al. (2008) Counter et al. (2007) Patil et al. (2006b)	Ferrochelatase inhibition: 10 μM Pb (30 min incubation) in rat liver mitochondria Increased ZPP: Blood Pb levels > 10 μg/dL
Ferrochelatase inhibition (biological plausibility)		
Consistent associations between blood Pb levels or Pb exposure and inhibition of ferrochelatase activity in epidemiologic studies of children and occupationally-exposed adults	Counter et al. (2009) Counter et al. (2008) Counter et al. (2007) Mohammad et al. (2008) Patil et al. (2006b) Ademuyiwa et al. (2005)	Children: Majority of concurrent blood Pb levels 10–20 μg/dL Adults: Majority of concurrent blood Pb levels >20 μg/dL
Decreased Hb, a direct marker of decreased heme synthesis (biological plausibility and coherence of MOA)		
Toxicological evidence: Decreased Hb in animals with relevant Pb exposures that is coherent with evidence in humans	Baranowska-Bosiacka et al. (2009) Lee et al. (2005) Marques et al. (2006) Sharma et al. (2010) Simsek et al. (2009)	Adult animals: Blood Pb levels 1.7–7.1 μg/dL after 15 days-9 months Pb exposure
Epidemiologic evidence[b]: Association between blood Pb levels and decreased Hb found in children, with consideration for potential confounding by age, sex, mouthing behavior, anemia, dairy product consumption, maternal age, education, employment, marital status, family structure, socioeconomic status.	Queirolo et al. (2010)	Children: Majority of concurrent blood Pb < 15 μg/dL
Epidemiologic evidence[b]: Association observed in other studies in children with limited or no consideration for potential confounding	Shah et al. (2010) Olivero-Verbel et al. (2007) Riddell et al. (2007)	
Epidemiologic evidence[b]: Multiple epidemiologic studies of adults observed decreases in Hb	Karita et al. (2005) Khan et al. (2008) Patil et al. (2006b) Ukaejiofo et al. (2009)	Adults: Majority of blood Pb > 20 μg/dL (occupational exposures)
ALAD inhibition (coherence of MOA)		
Toxicological evidence: A small, but consistent toxicology database indicates decreased heme synthesis in rodents with relevant Pb concentrations and routes of exposure	Rendón-Ramirez et al. (2007) Lee et al. (2005) Gautam and Flora (2010)	Drinking water exposures of 500–5,000 ppm, for 15–30 days as adults (Gautam and Flora, 2010; Rendón-Ramirez et al., 2007); oral exposures of 25 mg/kg BW once a week for 4 weeks (Lee et al., 2005)
Epidemiologic evidence[b]: Cross-sectional studies that considered potential confounding by age, sex, urban/rural residence, height, weight, body mass index found associations with lower ALAD activities in children	Ahamed et al. (2007) Ahamed et al. (2006)	Mean blood Pb levels <10 μg/dL
Epidemiologic evidence[b]: Concurrent blood Pb level associated with lower ALAD and higher ZPP in adults with consideration for potential confounding by age, sex, smoking status, and alcohol use	Wang et al. (2010)	Mean concurrent blood Pb level: 6.7 μg/dL
Epidemiologic evidence[b]: Associations found in several studies, mostly in occupationally-exposed adults, that did not consider potential confounding	Children: Ahamed et al. (2005) Occupational: Ademuyiwa et al. (2005) Mohammad et al. (2008) Patil et al. (2006a) Patil et al. (2006b)	Children: Mean concurrent blood Pb level: 7.5 μg/dL Adults (occupational exposure): Majority of concurrent blood Pb levels >20 μg/dL

Table 3 (*Continued*)

Description of key evidence with corresponding causal aspect(s) (biological plausibility and/or coherence)	Key references[a]	Pb exposure or blood Pb levels associated with effects
	Quintanar-Escorza et al. (2007)	
	Conterato et al. (2013)	
Ecological evidence: Decreased ALAD activity observed across taxa	Birds:	Birds:
	Gómez-Ramírez et al. (2011)	Blood Pb levels from 6->100 μg/dL
	Hansen et al. (2011)	
	Martínez-Haro et al. (2011)	
	Fish:	Fish:
	Schmitt et al. (2007)	Lower ALAD activity correlated with elevated blood Pb concentrations in wild caught fish from Pb-zinc mining areas (Schmitt et al., 2007; Schmitt et al., 2005); ALAD activity in brown trout linked to Pb accumulation on gills and in liver in a 23 day exposure to runoff water from a shooting range (15 to 46 μg/L) (Heier et al., 2009)
	Schmitt et al. (2005)	
	Heier et al. (2009)	
	Invertebrates:	Invertebrates:
	Oligochaetes:	Oligochaetes: 50% ALAD inhibition at 23 to 29 μg/L (48 h nominal exposure in aquaria)
	Aisemberg et al. (2005)	
	Bivalves:	Bivalves: positive correlation between increased tissue Pb levels and ALAD inhibition in field-collected bivalves (0.38–3.50 μg/g dry weight)
	Kalman et al. (2008)	
	Company et al. (2011)	
	Mammals and additional birds, fish, and invertebrates:	
	Eisler (2000)	

[a] The majority of reviewed studies were published from 2006 to 2011 with older studies supporting MOA.
[b] There is uncertainty in many studies, particularly for concurrent blood Pb levels, concerning the Pb exposure level, timing, frequency, and duration contributing to the observed effects and blood Pb levels measured.

hematological effects in the 2013 ISA for Pb (U.S. EPA, 2013a), specifically regarding effects on ALAD and heme synthesis associated with exposure to Pb (Table 2). Effects of Pb on hematological endpoints have been reported in epidemiologic studies, animal toxicological studies and in biota from aquatic and terrestrial habitats for several decades, and the underlying key events in the MOA are well characterized. The proteins involved in heme synthesis that are affected by Pb are highly conserved across species, which may account for the common response seen in health and ecological studies. This evolutionarily conserved response to Pb is likely the result of the competition of Pb with the necessary metal cofactors in the proteins involved in heme synthesis.

3.2. Reproductive and developmental effects

Reproductive toxicity of Pb was reported in the first Agency review of the NAAQS (U.S. EPA, 1977) and continues to be characterized in many species (U.S. EPA, 1986, 2006a,b, 2013a). In the 2013 ISA for Pb, coherence among findings in ecological receptors, humans, and laboratory animals was identified for some of the reproductive and developmental effects of Pb exposure (U.S. EPA, 2013a) (Table 4). Among reproductive and developmental health outcomes in humans and animal toxicology, relationships between Pb and both timing of puberty onset and male reproductive function were determined to be causal in the 2013 ISA for Pb (U.S. EPA, 2013a) (Table 2). Evidence for effects on other reproductive endpoints, such as birth outcomes and female reproductive function, is more limited. For ecological receptors, the overall cumulative evidence in invertebrates and vertebrates in terrestrial and aquatic habitats supported a causal relationship between Pb exposure and reproductive and developmental effects. This determination was based on a variety of endpoints associated with organism fecundity. Sensitive invertebrate taxa (e.g., gastropods, amphipods, cladocerans, rotifers) were identified in which reproductive and/or developmental effects were observed with laboratory exposures at or near concentrations of Pb measured in the environment (U.S. EPA, 1986, 2006a,b, 2013a).

3.2.1. Delayed maturation

Both epidemiologic and laboratory animal studies reviewed in the ISA for Pb (U.S. EPA, 2013a) support a relationship between Pb exposure and delayed puberty onset. Epidemiologic studies have demonstrated an inverse relationship of Pb on pubertal development among both girls and boys at low blood Pb levels. These studies were mostly cross-sectional, which do not allow for the study of temporality between Pb exposure and pubertal onset, nor do they consider the influence of past blood Pb levels. However, associations were also observed between blood Pb levels and delayed puberty in a longitudinal study of boys (Williams et al., 2010). Most of the studies had good sample sizes and controlled for some potential confounders. Nutritional information was rarely controlled, although this could be important especially in populations where malnutrition is prevalent. These observations of delayed onset of puberty in humans and laboratory animals are coherent with reports of Pb-associated maturational delays in a few aquatic and terrestrial organisms. Several ecotoxicological studies in invertebrates show Pb effects on maturation including prolonged development (aphids), delayed production of first offspring (amphipods), and increased generation time (nematodes) (Guo et al., 2009; Görür, 2007; Ringenary et al., 2007; Wang and Yang, 2007). Additionally, delayed metamorphosis following exposure to Pb in water or sediments has been reported for several species of frogs (Chen et al., 2006; Sparling et al., 2006).

Table 4
Summary of studies included in the 2013 ISA for Lead that provide mode of action (biological plausibility) and cross-species (coherence) evidence for the causal determination for reproductive and developmental effects.

Description of key evidence with corresponding causal aspect(s) (biological plausibility and/or coherence)	Key references[a]	Pb exposure or blood Pb levels associated with effects
Pb-induced oxidative stress is a key event in underlying MOA for reproductive effects (biological plausibility)		
Oxidative stress contributes to impaired sperm capacitation, sperm production, and sperm counts.	Male rodents: Hsu et al. (1998) Hsu et al. (1997)	Mean blood Pb levels 48.0–63.7 μg/dL
Co-treatment of Pb with antioxidants prevented Pb-induced effects on sperm count, sperm motility, and sperm viability in rodents.	Male rodents: Salawu et al. (2009) Shan et al. (2009) Madhavi et al. (2007) Rubio et al. (2006) Wang et al. (2006)	Drinking water exposure of 10,000 ppm for 8 weeks (Salawu et al., 2009); 20 mg/kg BW Pb for 6 weeks (Shan et al., 2009); 40 mg/kg BW Pb for 35 days (Madhavi et al., 2007); 0–24 mg/kg BW Pb for 35 days (Rubio et al., 2006); 2000 ppm Pb acetate drinking water (Wang et al., 2006)
Alterations in hormone levels, ratios, or biomarkers associated with sexual maturation (biological plausibility and coherence of MOA)		
Toxicological evidence: Pb-induced decrements in LH, estradiol, LH-releasing hormone, and IGF-1 observed in female rodents; IGF-1 treatment restored normal timing of puberty in rodents.	Female rodents: Dearth et al. (2002) Pine et al. (2006)	Mean blood Pb levels at breeding: 38–40 μg/dL
Toxicological evidence: Pb-induced decrements in FSH, serum testosterone, GnRH stimulation of LH release, LH-releasing hormone, and inhibin/FSH ratio observed in male rats and monkeys.	Male rats: Sokol et al. (1985) McGivern et al. (1991) Male monkeys: Foster et al. (1993)	Male rats: Effects observed in adult male rats after drinking water exposure to 1000 ppm, resulting in mean blood Pb level of 34 μg/dL (Sokol et al., 1985); effects observed in male offspring after gestational and/or lactational exposure (resulting in mean blood Pb level of 63 μg/dL and decreasing to <5 μg/dL at weaning) (McGivern et al., 1991) Male monkeys: Mean blood Pb levels 32–36 μg/dL before weaning and 19–26 μg/dL after weaning
Epidemiologic evidence[b]: Higher Pb levels associated with lower levels of FSH and inhibin B with mixed results for LH in girls. Higher Pb levels associated with lower levels of testosterone, FSH, and LH in boys.	Girls: Gollenberg et al. (2010) Tomoum et al. (2010) Boys: Tomoum et al. (2010)	Groups with concurrent blood Pb levels <10 μg/dL
Ecological evidence: Disrupted vitellogenin production (moths) and alterations in steroid hormone levels (fish, pigs)	Invertebrates: Moths: Shu et al. (2009) Fish: Catfish (*in vitro*): Chaube et al. (2010) Carp: Ramesh et al. (2009) Tilapia: Fırat et al. (2011) Mammals: Yu et al. (2005)	Invertebrates: Moths: Adult females reared on diets containing 25, 50 100, or 200 mg/kg exhibited decreasing vitellogenin with increasing Pb Fish: Catfish (in vitro): 12 and 24 h exposure of post-vitellogenic ovaries to 10–10,000 ug/L (nominal) (Chaube et al., 2010) Carp: 410 ug/L for 35 days (nominal) (Ramesh et al., 2009) Tilapia: 50 ug/L (nominal) for 4 days (Fırat et al., 2011) Mammals: Dietary exposure of 10 mg/kg for 120 days in pigs
Delayed maturation (coherence of effect)		
Toxicological evidence: Pb-induced delays in development and sexual maturation observed in female and male rodents. Female biomarkers include vaginal opening, age at first estrus, age at first vaginal plug formation, and age at first parturition; male biomarkers include prostate weight and testis weight.	Female rodents: Iavicoli et al. (2006) Iavicoli et al. (2004) Dearth et al. (2002) Dearth et al. (2004)	Female rodents: Effects observed in mice after gestational and lactational exposures to 0.06–20 ppm Pb in feed (Iavicoli et al., 2006; Iavicoli et al., 2004); effects observed in rats after gestational and lactational exposure to 12 mg/ml (Dearth et al., 2004; Dearth et al., 2002)

Table 4 (*Continued*)

Description of key evidence with corresponding causal aspect(s) (biological plausibility and/or coherence)	Key references[a]	Pb exposure or blood Pb levels associated with effects
	Male rodents: Ronis et al. (1998) Sokol et al. (1985)	Male rodents: Effects seen after exposures of 1000 to 6000 ppm Pb acetate in drinking water
Epidemiologic evidence[b]: Blood Pb levels associated with delayed puberty onset in girls (measured by Tanner staging, pubic hair development, breast development, and age of first menarche) and boys (measured by Tanner staging, pubic hair development, testicular size, and genital development) in mostly cross-sectional studies with control for potential confounding factors by inclusion of covariates such as age, body mass index, and/or education/SES.	Girls: Selevan et al. (2003) Tomoum et al. (2010) Naicker et al. (2010) Den Hond et al. (2011) Wu et al. (2003) Denham et al. (2005) Boys: Hauser et al. (2008) Williams et al. (2010) Tomoum et al. (2010)	Mean concurrent blood Pb levels <10 μg/dL
Ecological evidence: Maturational delays after Pb exposure observed in multiple species as demonstrated by increased time to metamorphosis (frogs), prolonged developmental stages (nematodes; fruit flies mosquitoes; aphids), prolonged generation time (nematodes), and time until first progeny (amphipods).	Amphibians: Frogs: Chen et al. (2006) Sparling et al. (2006) Invertebrates: Nematodes: Wang and Yang (2007) Guo et al. (2009) Fruit flies: Stamenkovic-Radak et al. (2008) Mosquitoes: Kitvatanachai et al. (2005) Aphids: Görür (2007) Amphipods: Ringenary et al. (2007)	Amphibians: Frogs: Time to metamorphosis delayed in northern leopard frog at 100 μg/L (Chen et al., 2006); time to metamorphosis delayed in southern leopard frog at 2360 mg/kg in sediment (Sparling et al., 2006) Invertebrates: Nematodes: 550, 16,000, and 41,000 μg/L for 3 days and generation time prolonged with increasing Pb (Wang and Yang, 2007); 500, 10,000 and 21,000 μg/L up to three days and generation time prolonged with increasing Pb (Guo et al., 2009) Fruit flies: 2200 μg/L and 9000 μg/L added to media-progeny of individuals reared in 9000 μg/L in media had significantly extended development time Mosquitoes: time to first emergence increased at 200 μg/L Aphids: prolonged generation time in aphids reared for several generations on plants watered with 870 μg/L (nominal) Amphipods: ≥118 mg/kg sediment (onset to reproduction significantly delayed in 60+ day multigenerational assay)
Sperm and male reproductive effects (coherence of effect)		
Toxicological evidence: Effects on sperm production, sperm quality (motility, viability, *in vitro* fertilization rates) and male reproductive organs (damage to seminiferous tubules, sperm abnormalities, acrosome integrity, and organ weights).	Male rodents: Sokol and Berman (1991) Sokol et al. (1985) Rubio et al. (2006) Anjum et al. (2010) Pillai et al. (2012) Dong et al. (2009) Biswas and Ghosh (2006) El Shafai et al. (2011) Murthy et al. (1995) Male rabbits: Moorman et al. (1998) Male monkeys: Singh et al. (1993) Foster et al. (1998)	Majority blood Pb levels >20 μg/dL after exposures (drinking water, oral capsule, oral gavage, i.p. injection) Several studies with blood Pb levels <10 μg/dL
Epidemiologic evidence[b]: Studies of occupationally exposed men showed negative effects on reproductive endpoints, including increased sperm head abnormalities, sperm DNA denaturation and hyploidy, decreased sperm motility and velocity, decreased sperm count, and decreased sperm viability.	Naha and Manna (2007) Naha and Chowdhury (2006) Hsu et al. (2009) Kasperczyk et al. (2008)	Concurrent blood Pb levels: ≥25 μg/dL

Epidemiologic evidence[b]: Detrimental effects on sperm observed among men at fertility clinics with lower Pb levels than those occupationally exposed, but there is a large degree of imprecision and inconsistency for some of these studies.	Telisman et al. (2007) Meeker et al. (2008) Mendiola et al. (2011)	Concurrent blood Pb levels: groups with ≤15 μg/dL
Ecological evidence: Effects on sperm production and quality across taxa (deer, Asian earthworms, rainbow trout, marine worms, fathead minnow)	Mammals: Deer: Reglero et al. (2009) Fish: Rainbow trout: Ruby et al. (1993) Fathead minnow: Weber (1993) Invertebrates: Asian earthworm: Zheng and Li (2009) Tubeworm: Gopalakrishnan et al. (2008)	Mammals: Deer: reduction in spermatozoa and acrosome integrity in deer from Pb-contaminated mining area compared to deer from a reference area Fish: Rainbow trout: 10 μg/L for 12 days decreased spermatocytes Fathead minnow: 500 μg/L for four weeks suppressed spermatocyte production Invertebrates: Asian earthworm: 1000–2,500 mg/kg (nominal) in soil 14 days altered sperm morphology Tubeworm: fertilization rate of *Hydroides elegans* reduced ~70% with sperm pretreated in 97 Pb/L for 20 min; EC_{50} for sperm toxicity 380 Pb/L

[a] The majority of reviewed studies were published from 2006 to 2011 with older studies supporting MOA.
[b] There is uncertainty in many studies, particularly for concurrent blood Pb levels, concerning the Pb exposure level, timing, frequency, and duration contributing to the observed effects and blood Pb levels measured.

3.2.2. Male reproductive function

Extensive epidemiologic and toxicological evidence supports an association between Pb and sperm/semen production, quality, and function (Table 4). Pb exposure affects sperm quality and function as well as damages male reproductive organs of laboratory mammals including rabbits and rodents (Nava-Hernandez et al., 2009; Moorman et al., 1998). Toxicological and epidemiologic studies support one another; epidemiologic studies in occupationally exposed men and some but not all studies of men from fertility clinics report associations of Pb exposure or blood Pb levels with decrements in a variety of sperm viability measures including decreased sperm count and sperm head abnormalities (Mendiola et al., 2011; Hsu et al., 2009; Kasperczyk et al., 2008; Meeker et al., 2008; Naha and Manna, 2007; Telisman et al., 2007; Naha and Chowdhury, 2006). However, the epidemiologic studies are limited by the high blood Pb levels in occupational cohorts and the potential selection bias in studies of men attending infertility clinics. Additionally, control for potential confounding factors, such as other workplace exposures, was not often performed. These findings are coherent with Pb-associated effects on sperm and male reproductive organs in wildlife from the ecological literature including deer, Asian earthworms, rainbow trout, marine worms and fathead minnow (Reglero et al., 2009; Zheng and Li, 2009; Gopalakrishnan et al., 2008; Ruby et al., 1993; Weber, 1993).

3.2.3. MOA for reproductive effects

Information on potential key events leading to these changes in maturation, puberty onset, and male reproductive effects is provided by studies that suggest Pb-induced changes to the hypothalamic–pituitary–gonadal (HPG) axis in humans and rodents and oxidative stress across multiple species. Further characterization of the sequence of key events for Pb acting as a potent endocrine disrupting compound is supported by findings that Pb disrupts the HPG axis through both oxidative stress and interference with metal cations responsible for secondary messenger systems and receptor ligand binding (Biswas and Ghosh, 2006; Rubio et al., 2006; Wiebe et al., 1988). The HPG axis functions to regulate circulating sex steroids and growth factors required for normal growth and development.

Insulin-like growth factor (IGF-1), inhibin B, gonadotropin releasing hormone (GnRH) pulsatility, serum follicle stimulating hormone (FSH), testosterone, estradiol, and luteinizing hormone (LH) have been shown to be affected by Pb exposure in rats (Dearth et al., 2002) and may contribute to Pb-associated changes in puberty timing or other reproductive and developmental endpoints including effects on sperm. Changes in HPG hormones may result from the Pb-induced inhibition of LH secretion (Bratton et al., 1994), reduction in the expression of the steroidogenic acute regulatory protein (Huang and Liu, 2004; Srivastava et al., 2004), and changes in IGF-1 (Pine et al., 2006; Dearth et al., 2002; Huseman et al., 1992). IGF-1 replacement therapy in laboratory rodents exposed to Pb restored the onset of puberty to its normal timing (Pine et al., 2006; Dearth et al., 2002). Pb-induced changes to hormone homeostasis observed in laboratory animals may also be due to altered ion status in essential signaling molecules or to oxidative stress. However, the evidence that is available is limited, and these key events are not consistently observed in all species or studies. In other vertebrates, fluctuations in hormone levels associated with Pb exposure have been reported in a few species including pigs (Yu et al., 2005) and fish (Fırat et al., 2011; Chaube et al., 2010; Ramesh et al., 2009). Some epidemiologic studies of delayed puberty have also reported differences in hormones related to blood Pb levels (Table 4).

Events leading to altered hormone homeostasis associated with Pb exposure in epidemiologic, animal toxicological, and ecological

studies represent a plausible sequence of key events in the MOA for reproductive and developmental effects. Studies in laboratory rodents have characterized a potential key event underlying the effects of Pb on sperm by showing that oxidative stress is associated with impaired sperm maturation, function, and sperm count. Sperm maturation through the process of capacitation is impaired in Pb-exposed laboratory animals leading to impaired spermatozoa function and metabolism, a process that is related to elevated ROS production in Pb-exposed rats' spermatozoa. These animals also had significantly decreased epididymal sperm counts. Pb-exposed male rats had early onset capacitation via a pathway involving elevated ROS generation; consequently, these Pb-exposed animals have functionally-impaired sperm showing premature acrosomal reaction that could potentially reduce the oocyte-penetrating capacity of the sperm (Hsu et al., 1997, 1998). Thus, effects of Pb on sperm function have been shown to be related to the key event of oxidative stress.

3.2.4. The cross-species approach: conclusions for reproductive and developmental effects of Pb

In general, coherence of reproductive and developmental effects was observed in wildlife studies and epidemiologic and animal toxicology studies for maturation endpoints, effects on sperm and the HPG axis. Causal relationships for reproductive and developmental effects in biota were supported by general findings in human health and animal toxicological studies for effects on fecundity, development, and hormone homeostasis (Table 2). In looking across species, several limitations to the available data were identified. Reproductive and developmental effects of Pb are widely reported in the ecotoxicological literature; however, no single endpoint in a single taxon has been extensively studied. Underlying mechanisms have not been thoroughly characterized for reproductive and developmental effects across species, especially in wildlife and biota. While both oxidative stress and alterations in the HPG axis may be involved in these effects across species, more data on key events in the MOA are needed.

3.3. Nervous system effects

The central nervous system of animals had been recognized as a target of Pb toxicity prior to the first Agency review of the NAAQS (U.S. EPA, 1977), and subsequent reviews have substantiated conclusions that Pb is a neurotoxicant in many organisms (U.S. EPA, 1986, 2006a, 2013a). The U.S. EPA concludes there is a causal relationship between Pb exposure and cognitive function decrements as well as attention decrements, impulsivity, and hyperactivity in children (Table 2) (U.S. EPA, 2013a). There is likely to be a causal relationship between Pb exposure and internalizing behaviors, conduct disorders, and motor function in children, with some uncertainty remaining because of the limited number of studies in children and inconsistent evidence in experimental animal models. There is likely to be a causal relationship between Pb exposure and neurobehavioral effects overall in terrestrial and freshwater organisms, based on evidence for effects on learning and behaviors such as decreased food consumption, prey capture ability, and motor control, which may decrease the overall fitness of the organism (Table 5). Some uncertainty remains for ecological effects, because evidence is available only for a few species and endpoints. Moreover, many of the reported ecological effects were observed at concentrations of Pb much higher than those routinely measured in the environment (U.S. EPA, 2013a). For both health and ecological effects, there is greater uncertainty regarding relationships of Pb exposure with auditory function, visual function, and neurodegenerative effects.

3.3.1. Cognitive function

The evidence for Pb-induced changes in oxidative stress and neuronal structure and function describe plausible key events mediating the effects of Pb on cognition (Table 5). Evidence for such effects with environmentally-relevant exposures to Pb through diet (Table 5), a major route of exposure across species, provides a strong basis for drawing coherence with effects on cognition observed across species. A robust evidence base links Pb exposure to decrements in cognitive function in children (Chandramouli et al., 2009; Miranda et al., 2009; Schnaas et al., 2006; Lanphear et al., 2005; Canfield et al., 2003; Wasserman et al., 1997; Bellinger et al., 1992; Dietrich et al., 1992). Common strengths of these studies that substantiate their findings include the longitudinal design with repeated assessments of blood Pb level and cognition throughout childhood, the use of well-validated instruments to measure cognitive function, and the examination of several other risk factors of cognitive decrements that could bias or confound associations with blood Pb level. Such confounding factors include socioeconomic status, maternal intelligence and education, parental caregiving quality, smoking exposure, and birth weight. Observations in children are coherent with findings of Pb-induced learning impairments in experimental rodents and monkeys (Cory-Slechta et al., 2010; Niu et al., 2009; McGlothan et al., 2008; Stangle et al., 2007; Altmann et al., 1993; Rice and Karpinski, 1988; Gilbert and Rice, 1987), as well as herring gull chicks and fish (Rice et al., 2011; Burger and Gochfeld, 2005). Strengthening the coherence of evidence across species, some studies in rodents and monkeys found impaired cognition with similar blood Pb levels (e.g., 13 and 15 μg/dL) as those measured in children (Table 5) and found impairments in similar domains, i.e., spatial memory and executive function.

3.3.2. Externalizing behaviors

Pb-related changes in neuronal structure, especially in the hippocampus, also support findings in multiple species indicating Pb-related changes in externalizing behaviors. Coherence among species is limited largely to findings for impulsivity in children, rodents, and monkeys. Several studies in children indicate blood Pb-associated attention decrements, impulsivity, and hyperactivity (Plusquellec et al., 2010; Chandramouli et al., 2009; Chiodo et al., 2004, 2007; Ris et al., 2004; Burns et al., 1999; Bellinger et al., 1994a). Many studies of externalizing behaviors had similar strengths as studies of cognition, described above. However, the strongest studies, as characterized by longitudinal design and examination of several potential confounding factors, found associations in populations with mean blood Pb levels of 6.8–14 μg/dL. While these blood Pb levels were defined in the ISA to be relevant to U.S. air-related Pb exposures, they are higher than those measured in most of the current U.S. population. Pb effects on impulsivity also were found in rodents and monkeys (Rossi-George et al., 2011; Brockel and Cory-Slechta, 1998, 1999; Gilbert and Rice, 1987; Rice and Gilbert, 1985). Similar to effects on cognition, impulsivity was induced in animals with blood Pb levels (e.g., 11 and 15 μg/dL) comparable to those in children. An additional strength of the studies of impulsivity was the examination of response inhibition, which also was observed to be impaired in children in association with higher blood Pb levels.

3.3.3. Neurotransmitter function to support effects on cognitive function, externalizing behaviors, internalizing behaviors, and motor function

Calcium plays a key role in regulating neurotransmitters, which mediate a broad array of nervous system effects from cognition and behavior to mood and motor function. Thus, the lines of evidence

Table 5
Summary of studies included in the 2013 ISA for Lead that provide mode of action (biological plausibility) and cross-species (coherence) evidence for the causal determination for nervous system effects.

Description of key evidence with corresponding causal aspect(s) (biological plausibility and/or coherence)	Key references[a]	Pb exposure or blood Pb levels associated with effects
Increased permeability of blood brain barrier (biological plausibility)		
Evidence of leaky cerebral vasculature, decreases in tight junction proteins, decreases in resistance across junctions, and increases in apparent diffusion coefficient in Pb-exposed rats.	Struzynska et al. (1997) Moorhouse et al. (1988) Wang et al. (2007) López-Larrubia and Cauli (2011)	Mean blood Pb levels 30–55 μg/dL with lactational and/or juvenile dietary Pb exposure in juvenile rats or lifetime dietary exposure in adult rats
Effects on neuronal structure and function (coherence of MOA)		
Toxicological evidence: Studies in rodents show effects of Pb exposure on impaired neurogenesis, neurite outgrowth, and synaptic plasticity.	Niu et al. (2009) Schneider et al. (2005) Verina et al. (2007)	Mean blood Pb levels 17–26 μg/dL with prenatal-juvenile or juvenile-only dietary exposure
Ecological evidence: Pb induced neural degradation or destruction of spinal neurons in multiple taxa. Pb-induced decreases in expression of neurogenesis genes and apoptosis of brain cells observed in zebrafish embryos.	Nematodes: Xing et al. (2009) Fish: African catfish: Adeyemo (2008) Zebrafish: Dou and Zhang (2011)	Nematodes: 500 μg/L for 6 h Fish: African catfish: 50–1000 μg/L for 4 or 8 weeks (nominal) Zebrafish: 50–70 μM in embryo medium from 0 to 6 days post hatch
Release or regulation of neurotransmitters (coherence of MOA)		
Toxicological evidence: Pb induced decreases in hippocampal GABA release, alterations in serotonin, and alterations in dopamine in rats.	Lasley and Gilbert (2002) Virgolini et al. (2008) Niu et al. (2009) Leasure et al. (2008) Cory-Slechta et al. (1992) Gedeon et al. (2001) Devi et al. (2005) Leret et al. (2002) Nowak et al. (2008)	Mean blood Pb levels 10–42 μg/dL with prenatal only, prenatal-lactational, prenatal-juvenile, lactational only, or juvenile only dietary exposure
Ecological evidence: Pb induced alterations in GABA motor neurons in nematodes and brain serotonin concentrations in fish species.	Invertebrates: Du and Wang (2009) Fish: Fathead minnow: Weber et al. (1991) Rainbow trout: Rademacher et al. (2003) Sloman et al. (2005)	Invertebrates: 500 μg/L for 24 h Fish: Fathead minnow: 1000 ug/L for 28 days Rainbow trout: trout were fed a minnow injected with 1.5 mg/kg daily for 2 weeks (Rademacher et al., 2003); 46 or 325 μg/L for 48 h (Sloman et al., 2005)
Cognitive function (coherence of effect)		
Toxicological evidence: Learning impairments in Pb-exposed rodents and monkeys demonstrated as impaired spatial memory, working memory, executive function, ability to learn response sequences, and associative ability.	Rodents: Stangle et al. (2007) Cory-Slechta et al. (2010) Altmann et al. (1993) McGlothan et al. (2008) Niu et al. (2009) Monkeys: Gilbert and Rice (1987) Rice and Karpinski (1988)	Rodents: Mean blood Pb levels 13–26 μg/dL with prenatal-lactational, prenatal-juvenile, prenatal-lifetime, or lactational-juvenile dietary Pb exposure Monkeys: Mean blood Pb levels 15 and 25 μg/dL with lifetime dietary Pb exposure from birth
Epidemiologic evidence[b]: Higher blood and tooth Pb levels associated with decrements in intelligence quotient, spatial memory, executive function, and academic performance in children. Studies followed children over time and adjusted for potential confounding by socioeconomic status, maternal intelligence quotient, maternal education, parental caregiving quality, birth weight, and smoking.	Canfield et al. (2003) Bellinger et al. (1992) Lanphear et al. (2005) Miranda et al. (2009) Dietrich et al. (1992) Schnaas et al. (2006) Wasserman et al. (1997) Chandramouli et al. (2009)	Mean blood Pb levels 3–16 μg/dL measured prenatally, in early childhood, concurrently with cognitive function, and averaged over the lifetime. Mean tooth Pb levels 3.3 and 6.2 μg/g at ages 6–8 years.

Table 5 (*Continued*)

Description of key evidence with corresponding causal aspect(s) (biological plausibility and/or coherence)	Key references[a]	Pb exposure or blood Pb levels associated with effects
Ecological evidence: In a limited number of studies, Pb-induced learning effects demonstrated by decreased recognition of caretakers (herring gull chicks) and diminished response to visual contrast (fish).	Birds: Herring gull chicks: Burger and Gochfeld (2005) Fish: Zebrafish: Rice et al. (2011)	Birds: Herring gull chicks: injection of 100 mg/kg bodyweight (producing feather Pb concentrations relevant to concentrations in wild gulls) Fish: Zebrafish: embryos exposed nominally (2.0 or 6.0 μg/L) to 24 h post-fertilization and then subsequently tested as adults
Motor function (coherence of effect)		
Toxicological evidence: Effects on endurance, balance, and coordination found in rodents with dietary exposure; inconsistent effects with exposure routes (e.g., injection) and concentrations may not be relevant to current U.S air-related Pb exposures.	Effects found: Leasure et al. (2008) Stangle et al. (2007) Not relevant exposures: Kishi et al. (1983) Grant et al. (1980) Overmann (1977)	Effects found with mean blood Pb levels of 10 and 31 μg/dL with prenatal-lactational or lactational-juvenile dietary exposure. Not relevant exposures: mean blood Pb levels 59 and 174 μg/dL with lactational or juvenile intubation of Pb or 67 μg/dL with dietary exposure
Epidemiological evidence[b]: Higher blood Pb levels associated with poorer dexterity, movement speed, balance, and agility in a few different cohorts of children. Studies followed children over time and adjusted for potential confounding by socioeconomic status, parental education, maternal intelligence quotient, and parental caregiving quality.	Ris et al. (2004) Dietrich et al. (1992) Wasserman et al. (2000)	Mean blood Pb levels 5–12 μg/dL measured neonatally, concurrently with motor function, or averaged over the lifetime
Ecological evidence: Pb-induced decrements in motor function found in multiple species. Observed effects include decreased motility (nematodes); decreased walking (gull chicks); and decreased prey capture ability (fathead minnow).	Invertebrates: Nematodes: Wang and Yang (2007) Wang and Xing (2008) Birds: Herring gull chicks: Burger and Gochfeld (2005) Fish: Fathead minnow: Mager et al. (2010)	Invertebrates: Nematodes: 500 μg/L for 3 days (nominal) (Wang and Yang, 2007); 500 μg/L for 1 day (nominal) (Wang and Xing, 2008) Birds: Herring gull chicks: injection of 100 mg/kg bodyweight (producing feather Pb concentration relevant to concentrations in wild gulls) Fish: Fathead minnow: 10 day old larvae hatched from eggs of adult fish exposed to 120 μg/L for 300 days
Pb was associated with behavioral effects in multiple species. Coherence was found between epidemiologic and toxicological studies, but these endpoints lack clear homology with endpoints in ecological literature. (coherence of effect)		
Toxicological evidence: Impulsivity (demonstrated as impaired response inhibition), depression-like behavior, and emotionality observed in rodents and monkeys with Pb exposure.	Rodents: Dyatlov and Lawrence (2002) Rossi-George et al. (2011) Brockel and Cory-Slechta (1999) Brockel and Cory-Slechta (1998) Monkeys: Gilbert and Rice (1987) Rice and Gilbert (1985)	Rodents: Mean blood Pb levels 11–33 μg/dL with prenatal-lifetime, lactation only, or juvenile only dietary exposure Monkeys: Mean blood Pb levels 15 and 25 μg/dL with lifetime dietary exposure from birth
Epidemiologic evidence[b]: Higher blood Pb or tooth Pb level associated with attention decrements, impulsivity, hyperactivity, aggressive, antisocial, or delinquent behavior, withdrawn behavior, and symptoms of depression and anxiety in children. Many studies followed children over time and adjusted for potential confounding by socioeconomic status, maternal education, and parental caregiving quality.	Burns et al. (1999) Bellinger et al. (1994a) Bellinger et al. (1994b) Chiodo et al. (2007) Chiodo et al. (2004) Plusquellec et al. (2010) Chandramouli et al. (2009) Ris et al. (2004) Fergusson et al. (2008) Fergusson et al. (1993) Wright et al. (2008)	Mean blood Pb levels 5–14 μg/dL measured prenatally, in early childhood, concurrently with behavior, or averaged over the lifetime Mean tooth Pb levels 3.3 and 6.2 μg/g at ages 6–8 years

Ecological evidence: Behavioral effects with Pb exposure demonstrated in multiple species, including alterations in locomotion behaviors (nematodes); feeding behaviors (snails; pigs, lizards); posturing behaviors (lizards); erratic behavioral thermoregulation and food begging (herring gull chicks); and hyperactivity (wrasse).	Invertebrates: Nematodes: Wang and Xing (2008) Snails: Ebenso and Ologhobo (2009) El-Gendy et al. (2011) Mammals: Pigs: Yu et al. (2005) Reptiles: Western fence lizards: Salice et al. (2009) Birds: Herring gull chicks: Burger and Gochfeld (2005) Fish: Wrasse: Giusi et al. (2008)	Invertebrates: Nematodes: 500 μg/L for 1 day (Decreased head thrashes and body bends) Snails: Food consumption decreased with increasing Pb in diet from 0 to 1344 mg/kg for 12 weeks (Ebenso and Ologhobo, 2009); snail feeding rates depressed in 3 week dietary exposure of 50 to 15,000 mg/kg dry weight (nominal) (El-Gendy et al., 2011) Mammals: Pigs: Food intake decreased with 10 mg/kg in feed for 120 days (resulting in blood Pb level of 2.1 μg/dL) Reptiles: Western fence lizards: post-dose observations following 60-day dietary exposure to 10 or 20 mg/kg per day Birds: Herring gull chicks: Injection of 100 mg/kg bodyweight (producing feather Pb concentration relevant to concentrations in wild gulls) Fish: Wrasse: 400 or 1600 μg/L (nominal) for one week in seawater

[a] The majority of reviewed studies were published from 2006 to 2011 with older studies supporting MOA.
[b] There is uncertainty in many studies, particularly for concurrent blood Pb levels, concerning the Pb exposure level, timing, frequency, and duration contributing to the observed effects and blood Pb levels measured.

demonstrating the effects of Pb on disruption of calcium homeostasis as well as alterations in neurotransmitter regulation and release describe a plausible sequence of key events in the MOA for the nervous system effects of Pb. Pb exposure has been shown to affect gamma-aminobutyric acid (GABA), serotonin, and dopamine, with effects on GABA found in nematodes and experimental rodents (Du and Wang, 2009; Lasley and Gilbert, 2002) and effects on serotonin found in experimental rats and also in fathead minnow and rainbow trout (Virgolini et al., 2008; Sloman et al., 2005; Rademacher et al., 2003; Weber et al., 1991). These effects were demonstrated by several different investigators and often with blood Pb levels (means 10–27 μg/dL, see Table 5) considered relevant to U.S. air-related exposures. Pb was found to induce changes in GABA motor neurons in nematodes (Du and Wang, 2009), which may explain the Pb-associated decrements in motor function in nematodes (Wang and Xing, 2008). Pb exposure also has been associated with decreased motor function in frogs (Herkovits and Perez-Coll, 1991), herring gull chicks (Burger and Gochfeld, 2005), fathead minnows (Mager et al., 2010), and in children (Ris et al., 2004; Wasserman et al., 2000; Dietrich et al., 1992). The evidence in children is based on several of the same cohorts in which Pb-associated effects on cognition and externalizing behaviors were observed. In rodents, Pb exposure has not consistently affected motor function as assessed by endurance, balance, and coordination. Where effects have been found, the implications of findings are limited by Pb exposures with routes (i.e., intraperitoneal injection) and concentrations that may not be relevant to current U.S. air-related exposures (Leasure et al., 2008; Stangle et al., 2007; Kishi et al., 1983; Grant et al., 1980; Overmann, 1977).

Pb-induced changes in GABA and serotonin also may provide a mechanistic explanation for the Pb-related effects observed on externalizing behaviors as described above as well as the associations observed in children between tooth or blood Pb level and internalizing behaviors such as withdrawn behavior and symptoms of depression and anxiety (Plusquellec et al., 2010; Chiodo et al., 2004; Burns et al., 1999; Bellinger et al., 1994a). The supporting evidence in children was provided by both prospective studies and cross-sectional studies that concurrently measured blood Pb and behavior. Although the temporal sequence between Pb exposure and changes in behavior cannot be determined in the cross-sectional studies, findings from both prospective and cross-sectional studies are substantiated by the well-validated instruments to assess behavior and examination of several potential confounding factors as described earlier for cognitive function. Coherence is limited to findings in a few studies of rodents that Pb exposure induced depression-like behavior and emotionality (Beaudin et al., 2007; Dyatlov and Lawrence, 2002). Other studies reported Pb-related increases in emotionality and depression-like behavior in rodents and rhesus monkeys (Moore et al., 2008; de Souza Lisboa et al., 2005; Stewart et al., 1996) but have limited implications because Pb exposure concentrations and/or a route of exposure in these studies were not considered to be relevant to current U.S. air-related exposures. Whether these changes are coherent with the Pb-related changes in behavior observed in ecological biota is not clear. Across multiple species, Pb induced changes in feeding behaviors, avoidance responses, decreased ability of an organism to capture prey or escape predators, and posturing behaviors (El-Gendy et al., 2011; Mager et al., 2010; Ebenso and Ologhobo, 2009; Salice et al., 2009; Wang and Xing, 2008; Burger and Gochfeld, 2005; Yu et al., 2005). In general, behavioral responses to Pb were highly variable in biota with some studies showing no effects even at high concentrations of Pb (U.S. EPA, 2013a).

Dopamine is another neurotransmitter that integrates function in the hippocampus, prefrontal cortex, and nucleus accumbens of the brain to mediate cognitive function. In rodents, Pb consistently has been shown to alter dopamine release in these regions (Niu et al., 2009; Leasure et al., 2008; Nowak et al., 2008; Virgolini et al., 2008; Devi et al., 2005; Leret et al., 2002; Gedeon et al., 2001; Cory-Slechta et al., 1992) and decrease long-term potentiation (Niu et al., 2009), which is a major cellular event underlying synaptic plasticity, learning, and memory. Such changes may provide another explanation for the Pb-induced changes in cognitive function observed across species.

3.3.4. MOA for nervous system effects

The causal determinations made for both health and ecological effects regarding the nervous system effects of Pb exposure are supported by evidence describing key events in underlying MOAs such as changes in brain neuroanatomy and neurotransmitter function. Such changes were observed across various species, which may provide an explanation for the coherence of Pb-associated effects on cognitive function and motor function observed in humans, experimental animal models, and ecological taxa. This MOA evidence also supports changes in behavior observed across species in relation to Pb exposure, although the specific endpoints affected in humans and laboratory animals lacked clear homology with effects in ecological biota, particularly invertebrates.

Although examined primarily in laboratory rats, the effects of Pb exposure on increasing permeability of the blood–brain barrier (López-Larrubia and Cauli, 2011; Wang et al., 2007; Dyatlov et al., 1998; Struzynska et al., 1997; Moorhouse et al., 1988; Sundstrom et al., 1985) represent an early key event in the MOA for the Pb-related nervous system effects observed across species. Coherence is found among experimental animal models including rodents, nematodes, and fish for the effects of Pb on neuronal structure and function (e.g., impaired neurogenesis, synaptic plasticity, neurite outgrowth) (Dou and Zhang, 2011; Neal et al., 2010; Niu et al., 2009; Xing et al., 2009; Adeyemo, 2008; Hu et al., 2008; Verina et al., 2007; Schneider et al., 2005, 2003), with limited evidence in rats linking such changes to Pb-induced oxidative stress (Abdel Moneim et al., 2011; Hu et al., 2008).

3.3.5. The cross-species approach: conclusions for nervous system effects of Pb

In describing the weight of evidence for health and ecological effects, the 2013 ISA for Pb noted the coherence of evidence across species for the effects of Pb on learning as well as key events in the MOA such as changes in neuronal architecture and neurotransmitter function (U.S. EPA, 2013a). However, causal determinations were not strongly influenced by the cross-species coherence of effects and common MOAs. The coherence of evidence in children and in laboratory rodents and monkeys was sufficient to support a causal relationship for Pb with cognitive function as well as attention, impulsivity, and hyperactivity. In the ecological evidence base, there was uncertainty in a relationship between Pb exposure and neurobehavioral effects related to findings in a limited number of species and endpoints and higher than environmentally-relevant Pb exposures. This uncertainty could not be addressed by drawing from information in the health literature. Likewise, there was uncertainty in the health evidence base for relationships of Pb exposure with internalizing behaviors and motor function in children. This uncertainty was due to the lack of clear biological plausibility from toxicological evidence, which could not be addressed by drawing from the ecological literature. In ecological taxa, evidence for such effects was limited or not available for analogous endpoints.

3.4. Biological systems with less evidence for coherence

Some of the health outcomes and corresponding causal determinations reported in the 2013 ISA for Pb (U.S. EPA, 2013a) are supported by a substantial body of human health literature with little or no evidence in biota for these effects (Table 2). The cross-species approach described above focuses on a subset of the wide ranging effects of Pb documented in humans, laboratory animals and wildlife, for which there was sufficient evidence available to assess coherence of effects and underlying modes of action. Most studies of ecological endpoints are not designed to address mode of action directly, and some endpoints of Pb toxicity are not commonly assessed in wildlife. For example, based on findings in humans and laboratory animal toxicological models, a causal relationship is determined for Pb and cardiovascular effects (U.S. EPA, 2013a). However, few studies in wildlife assess cardiovascular fitness, and no data on this endpoint was identified in the ISA for Pb. In addition, evidence in humans and laboratory animals is suggestive of a relationship between Pb exposure and renal dysfunction. In contrast, with the exception of a laboratory study in fish (Patel et al., 2006), studies that reported effects of Pb on kidney function in wildlife were not identified in the ISA for Pb (U.S. EPA, 2013a). Further, the ISA for Pb determined a likely to be causal relationship for immune effects, based on evidence for atopic and inflammatory responses and decreased host resistance in humans and laboratory rodent models (U.S. EPA, 2013a). However, such information on Pb is limited in terrestrial and aquatic biota. Immunotoxic responses of a suite of heavy metals including Pb have been reported in marine mammals (Cámara Pellissó et al., 2008). Markers of oxidative damage and antioxidant activity have been observed in field studies in a wide range of species in aquatic and terrestrial environments where Pb (along with other chemicals) is present and also following laboratory exposures. However, these are more general markers of physiological stress and cannot be attributed directly to immune modulation.

4. Conclusions

In the 2013 ISA for Pb (U.S. EPA, 2013a), EPA evaluated evidence from various organisms for common effects and key events in MOAs in the context of a larger causal framework to form causal determinations for health and ecological effects of Pb exposure. By considering the modified Bradford Hill aspects for causality, specifically coherence and biological plausibility, evidence for the effects of Pb on common endpoints and underlying key events in the MOAs were used to support, in part, the causal determinations for hematological effects, developmental and reproductive effects, and nervous system effects in terrestrial and aquatic biota and humans (Table 2). This cross-species and transdisciplinary approach is also useful for identifying data gaps in the characterization of the sequence of events from early molecular and cellular key events to effects at the organ and system-level. Commonalities in effects and underlying key events in the MOA among human, animal toxicological, and ecological studies strengthened biological plausibility for hematological effects. Some reproductive and developmental effects were common across humans and ecological receptors; however, findings from human and rodent studies were unable to address uncertainties in the ecological literature for nervous system effects. Other effects such as cardiovascular, immune, and renal responses to Pb are

documented in the epidemiologic and animal toxicological literature but are not commonly assessed in wildlife.

As demonstrated for Pb, the utility of a cross-species approach in the characterization of toxicity of an environmental agent is maximized when data on comparable effects are available across species. Pb effects in humans and animal toxicological models may be applicable in the interpretation of ecological observations and vice-versa. Thus, although a cross-species approach may not be applied to all environmental agents or for all organ systems, the joint consideration of epidemiologic, animal toxicological, and ecological disciplines for the evaluation of policy-relevant science for the NAAQS could be applied in other regulatory settings where data are available to identify possible effects of common exposures between humans and biota.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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