Taylor & Francis
Taylor & Francis Group

Journal of Toxicology and Environmental Health, Part B
Critical Reviews

ISSN: 1093-7404 (Print) 1521-6950 (Online) Journal homepage: https://www.tandfonline.com/loi/uteb20

Hearing loss, lead (Pb) exposure, and noise: a sound approach to ototoxicity exploration

Krystin Carlson & Richard L. Neitzel

To cite this article: Krystin Carlson & Richard L. Neitzel (2018) Hearing loss, lead (Pb) exposure, and noise: a sound approach to ototoxicity exploration, Journal of Toxicology and Environmental Health, Part B, 21:5, 335-355, DOI: 10.1080/10937404.2018.1562391

To link to this article: https://doi.org/10.1080/10937404.2018.1562391

Published online: 21 Jan 2019.

JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH, PART B
2018, VOL. 21, NO. 5, 335–355
https://doi.org/10.1080/10937404.2018.1562391

Taylor & Francis
Taylor & Francis Group

Hearing loss, lead (Pb) exposure, and noise: a sound approach to ototoxicity exploration

Krystin Carlson and Richard L. Neitzel

Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI, USA

ABSTRACT

To determine the state of the research on ototoxic properties of Pb, evaluate possible synergistic effects with concurrent noise exposure, and identify opportunities to improve future research, we performed a review of the peer-reviewed literature to identify studies examining auditory damage due to Pb over the past 50 years. Thirty-eight studies (14 animal and 24 human) were reviewed. Of these, 24 suggested potential ototoxicity due to Pb exposure, while 14 found no evidence of ototoxicity. More animal studies are needed, especially those investigating Pb exposure levels that are occupationally and environmentally relevant to humans. Further investigations into potential interactions of Pb in the auditory system with other hazards and compounds that elicit ototoxicity are also needed in animal models. To better assess the effects of Pb exposure on the human auditory system and the possibility of a synergism with noise, future epidemiological studies need to carefully consider and address four main areas of uncertainty: (1) hearing examination and quantification of hearing loss, (2) Pb exposure evaluation, (3) noise exposure evaluation, and (4) the personal characteristics of those exposed. Two potentially confounding factors, protective factors and mixtures of ototoxicants, also warrant further exploration.

KEYWORDS

Ototoxicity; hearing loss; lead (Pb); noise; exposure mixtures

Introduction

A wide variety of adverse social, psychological, occupational, and educational outcomes stem from one disease process: hearing loss (HL) (Seidman and Standring 2010). HL is the third most disabling global disease (World Health Organization 2008), with about 466 million sufferers globally (World Health Organization 2018). The increasingly high prevalence of adult-onset HL in developing nations (Stevens et al. 2013) is due at least in part to occupational noise exposures (Nelson et al. 2005). While HL is often only thought of as a disease of the elderly, HL affects those of all ages, and is especially damaging to the quality of life and academic and social performance in children, even when cases are mild (Tharpe et al. 2009, Wake et al. 2004). Once damaged, the sense of hearing cannot be fully restored. Even with our furthered understanding of ototoxicants and improvements in deciphering the mechanism behind noise-induced HL, workers continue to suffer from occupational HL at alarming rates (Masterson et al. 2016). This may be due to a combination of potentially ototoxic chemicals in the workplace alongside poor levels of compliance with programs to protect workers' hearing (Rabinowitz et al. 2018); many traditional challenges to occupational HL prevention continue to threaten workers' hearing health today (Kerr et al. 2017).

A number of types of substances have demonstrated ototoxic properties, including organic solvents, certain medications, asphyxiants, metals (Campo, Morata, and Hong 2013), and pesticides (Mac Crawford et al. 2008). Of the various metals linked to ototoxicity, lead (Pb) has been most extensively examined, though evidence and mechanisms for Pb-induced damage still have not been fully elucidated. Pb ototoxicity is more heavily researched than other toxicant metals (e.g. Cd or Hg); however, the impacts of Pb on the auditory system have not been as extensively studied as are its well-known effects on the nervous (Mason, Harp, and Han 2014) and cardiovascular systems (Solenkova et al. 2014), and, at high doses, on the hepatic and renal systems (Goswami, Gachhui, and Bandopadhyay 2005). Pb is ubiquitous in many occupational and community settings due to its main current industrial purpose,

CONTACT Richard L. Neitzel rneitzel@umich.edu Department of Environmental Health Sciences, University of Michigan, 1415 Washington Heights 6611 SPH I, Ann Arbor, MI 48109

use in Pb-acid storage batteries, and environmental contamination from past uses in gasoline, plumbing, and paint. Given these factors, a better understanding of combined exposures to ototoxic agents in settings where vulnerable populations may be exposed is essential for developing plans to prevent HL.

In the past, HL was traditionally associated with age or noise exposures. Today, research has demonstrated that chemicals such as styrene and aminoglycosides contribute to auditory damage as well (Morata et al. 2011, Schacht, Talaska, and Rybak 2012). Additionally, epidemiological studies have indicated that exposures to heavy metals, including arsenic, cadmium, Pb, manganese, and mercury, may be associated with an increased risk of HL (Anniko and Sarkady 1978, Rybak 1992). These toxic metals cross the blood-brain barrier (Karri, Schuhmacher, and Kumar 2016) and can also cross into the inner ear and cochlea. Significant increases in toxic metals in the cochlear tissues of animals treated with arsenic and Pb have been established (Carlson, Schacht, and Neitzel 2018, Li et al. 2017).

Exposures to hazardous chemical mixtures represent an important area in risk assessment toxicology, as well as occupational health research (Simmons 1995). The US National Institute for Occupational Safety and Health (NIOSH) has highlighted the need for further research examining the relationship of metals to HL, especially those occurring in mixtures (Morata 2003). However, the impacts of Pb in combination with other hazards on the auditory system have not been extensively studied. Improvements in our understanding of the role of chemical agents causing HL are critical to preventing HL and identifying vulnerable workers in industries with multiple ototoxic exposures, as well as vulnerable communities and populations. As with Pb, noise is also ubiquitous in occupational and community settings (Lewis, Gershon, and Neitzel 2013), and interactions between physical and chemical hazards in toxicology are underappreciated (Rider et al. 2014).

Reviews of Pb-induced ototoxicity are few and have not included a thorough examination of study methodologies evaluating ototoxicity (Araki et al. 2000, Johnson and Morata 2010a, Otto and Fox 1993, Repko and Corum 1979). A recent paper briefly reviewed a wide variety of divalent metals for ototoxic properties (Roth and Salvi 2016). However, that review focused on delineating potential mechanisms, and not on identifying opportunities for improving the design of future studies, as our review does. Another recent publication examining hearing impairment caused by occupational chemical exposures did assess ototoxicity of Pb, but did not evaluate study methodologies, as our review does (Johnson and Morata 2010b). In order to extensively review and thoroughly analyze both the methods and results of past studies on metal toxicity, this review focuses solely on Pb exposures to non-developmental life-stages, both alone and in combination with other agents. To capture all related studies, we have included studies on animals and humans. Animal studies can provide evidence of harm unconfounded by other variables, so we discuss these findings first. In contrast, epidemiological studies explore combinations of variables in uncontrolled, real-life settings, and offer insights that are more directly relevant to human exposures. Examining both types of literature is critical to determining the degree to which Pb contributes to HL in humans.

Methods

Firstly, the authors wish to make a point about modern search engines. While these are convenient and advantageous in many ways, they fall short in overcoming some of the deficiencies of the English language, namely heteronyms. While two search engines, Google Scholar and PubMed, were employed in our review, the use of tabulated search criteria resulted in a more accurate and efficient search of the literature. PubMed searches usually returned relevant papers, whereas Google Scholar searches were highly confounded by the heteronym lead, as in a horse to water. The heteronym hearing, as in a legal setting, also obfuscated the topic of interest. Over 122,000 hits were returned using keywords “lead” AND “hearing loss” on Google Scholar. For this reason, we relied heavily on PubMed’s tabulated search criteria which, when refined, returned under 200 hits.

Our PubMed search used both keywords and Medical Subject Heading (MeSH) terms relating to topics of metals, ototoxicity, and Pb in both animal and human studies. Articles found were evaluated for references to additional literature of interest. Reports and review articles on general

ototoxicants (some of which addressed combinations of noise and chemical exposure) were also consulted to compile references (Cary, Clarke, and Delic 1997, European Agency for Health and Safety at Work 2009, Haider et al. 1990, Johnson and Morata 2010b, Prasher 2009, Rybak 1992).

To determine that a paper appropriately addressed HL, six MeSH terms were used in a Boolean search with an OR operator. These are listed below. Establishing the paper's relevance to Pb was more difficult. The NCBI MeSH term repository was essential for this aspect of the search due to the issues with heteronyms. The keyword "Pb" was also introduced to capture studies using the chemical abbreviation for "lead".

The main NCBI Boolean search used is below for reproducibility of our findings:

("Hearing"[Mesh]

OR "Hearing Loss, Mixed Conductive-Sensorineural "[Mesh]

OR "Hearing Loss"[Mesh]

OR "Persons With Hearing Impairments"[Mesh]

OR "Auditory Diseases, Central"[Mesh]

OR "Auditory Threshold"[Mesh])

AND

("Lead" [Mesh] OR "Pb")

Only literature published in English was considered in our search. Studies were excluded from this review if the outcome assessed was the vestibular system, conductive HL, or tinnitus; if auditory tests were used to measure cognition; if the goal of a study was reporting on the prevalence of hearing disorders; and if occupational or noise-induced HL were evaluated with no measure of Pb exposure. We next excluded all studies relating to developmental Pb exposures and auditory function including studies on animals *in utero* or in human children, though these studies have also shown inconsistent results (Buchanan, Counter, and Ortega 2011, Osman et al. 1999). While these studies may serve as additional evidence, the mechanisms and pathology are likely to be different, so we omitted these papers from our review. From the articles remaining, the references listed within the articles themselves, and literature found from previous reviews on ototoxicants, 38 papers were selected for inclusion in this review. These papers were grouped by the nature of research into studies performed with animals (n = 14) and human studies (n = 24).

From each selected study, key information was abstracted regarding the methodology used to perform and measure all predictor and outcome variables of interest, as well as study results. Each paper was analyzed for strengths and weaknesses, particularly with regards to the ototoxic mixtures and potential confounding factors. All studies were reviewed by a single author (KC).

Results

Animal experimental evidence

Thirteen studies in whole animals and one study in animal tissues were identified. Table 1 summarizes methods of animal study designs; the lengths of the studies, dosing regimens, and animal types are shown for comparison. Study results, along with noteworthy outcomes, are shown in Table 2. Tables 1 and 2 are arranged in chronological order overall, and alphabetical order for studies occurring in the same year. To summarize these studies, literature was classified into high dose studies in which animals were observed to suffer acute Pb poisoning symptoms and low dose studies which utilized chronic exposure conditions to evaluate lower Pb levels over time. Due to the differences in animals and the differences in time periods tested, no specific dose of Pb was used to delineate high from low. Overall, the majority (9 of 14 *in vivo* studies; the *in vitro* study did not support ototoxicity) support an ototoxic property of Pb. The animal studies identified in this review which provide evidence in favor of ototoxicity point to a neurological source of damage caused by Pb.

High Pb dose studies

Historic exploration into Pb ototoxicity involved high doses of Pb that caused frank toxicity. Of the thirteen *in vivo* studies, nine involved high doses of Pb. Eight of these caused animal death or weight loss due to Pb treatment, while one study used a high dose of Pb and euthanized animals two hours after treatment. Six of these eight studies established the strong possibility of alterations in the auditory system, and

Table 1. Features of thirteen *in vivo* with one *in vitro* Pb ototxicity toxicology studies

#	Study (by first author)	Animal	N	Age	Type of Pb	Dose	Study length	Exposure Assessment
	A) *IN VIVO* STUDIES							
1.	Gozdzik-Zolnierkiewicz and Moszynski (1969)	Guinea Pig	10 C; 40 Pb	300-350 g	1% $Pb/NO_{3/2}$	ip injections 300 mg/kg once a day	7 weeks	Estimated Pb levels: Control 25-330 µg/% Pb 310-420 µg/%
2.	Wilpizeski (1974)	Squirrel Monkey	12 Pb	Adult	Pb oxide (red Pb powder)	[preliminary treatments of peritoneal cavity 1 gram Pb foil (N=5); nasal spray of PbAc & red Pb] peroral injection 0.5 mL once a day	5 to 24 weeks; varied for individual monkey	BLL: Control 53 ± 51 µg/%Pb 338 ± 225 µg/%
3.	Takahashi et al. (1984)	Sprague-Dawley rats (male)	4 C; 6 Pb	11 weeks/ 230-320 g	0.1 mM PbAc	ip injection 1 mL/100g	Injected for four days. Measurements on days 1, 2, 3, 6, 9, and 11	Control > 10 µg/dL 267 ± 19 µg/dL
4.	Yamamura et al. (1984)	Guinea Pig – Hartley albino	10 C, 13 in 10 mg, 10 in 15 mg, 20 in 20 mg	9 weeks/ 320 g	1% PbAc	ip injection once a week of 0, 10, 15 or 20 mg	5 weeks	30 ± 14 µg/dL 154 ± 40 µg/dL 153 ± 46 µg/dL
5.	Yamamura et al. (1987)	Albino Hartley guinea pigs	10 C, 10 in 10 mg, 20 in 15 mg, 20 in 20 mg	9 weeks	1% water solution PbAc	ip injection once a week of 0, 10, 15 or 20 mg	5 weeks	4.9 ± 2.9 µg/dL 123 ± 39 µg/dL 102 ± 30 µg/dL 134 ± 52 µg/dL
6.	Yamamura et al. (1989)	Albino Hartley guinea pigs (male)	20 C; 19 in 2 wks; 20 in 4 wks; 20 in 5 wks	5 weeks of age or less	1% water solution PbAc	ip injection once a week; control; 20 mg/ week	Control; 2 weeks; 4 weeks;5 weeks	4.5 ± 2.4 µg/dL 80 ± 40 µg/dL 126 ± 19 µg/dL 124 ± 53 µg/dL
7.	Hotta et al. (1996)	Albino Hartley guinea pigs (male)	24 C;8 in 2 wk; 8 in 4 wk; 21 in 4 wk + noise	Over 5 weeks of age	1% solution PbAc	ip injection; 20 mg/week	Control; 2 weeks; 4 weeks (± noise);	3.2 + 1.5 µg/dL139 + 29 µg/dL 165 + 42 µg/dL
8.	Nagymajtenyi et al. (1996)	Wistar rats (male)	8 per group, 12 treatment variations of Pb dose and treatment time	10 weeks old	PbAc dissolved in distilled water; 0.1 mL/100g body weight	Gavage 80, 160, and 320 mg/kg body weight for 5 days a week	4 weeks; 8 weeks; 12 weeks	None given
9.	Tuncel et al. (2002)	2/NCR Guinea Pigs	5 per group oil C; ethanol C; PbAc-ethanol; TEPb-oil	-	PbAc and TEPb	ip injections PbAc 50 mg/kg; TEPb 42.7 mg/kg	Recordings followed 120 minutes after injection	tetraethyl Pb: 45.2-47.2 µg/dL PbAc: 45.2-47.2 µg/dL
10.	Fazakas et al. (2005)	Wistar rats	10 per group low-Pb; high-Pb; low-Pb +Al; high-Pb+Al; Pb-low +Hg; Pb-low+Hg+Al	12 weeks	PbAc dissolved in distilled water (with 5% v/v ethanol or 0.4 mg/kg mercuric chloride)	Gavage five days a week 80 mg/kg and 320 mg/kg	12 weeks	-

(*Continued*)

Table 1. (Continued).

#	Study (by first author)	Animal	N	Age	Type of Pb	Dose	Study length	Exposure Assessment
11.	Liu et al. (2011)	Wistar rats	C: 5 M/5F Pb: 5M/5F Pb+Cu 5M/5F	140 – 270 g	PbAc	Gavage of 4.0 mg/kg	30 days	Pb: M 1.2 ± 0.09 µg/dL, F 0.8 ± 0.02 µg/dL
12.	Carlson et al. (2018)	CBA/CaJ mice	C: 16 M 0.03-Pb: 8 M 1-Pb: 8 M 3-Pb: 16 M Pb+Cd: 9 M Pb+Noise: 14 M Pb+Cd+Noise: 7 M	5 weeks	PbAc from 2% w/v solution	In drinking water 0.03, 1, and 3 mM dissolved in Milli-Q water	Final ABR after 10 weeks and tissues collected after 11 weeks Pb	BLL (µg/dL): 0.03 mM Pb – 3 ± 0.4 1 mM Pb – 39 ± 5 3 mM Pb – 60 ± 7 3 mM Pb+Cd – 58 ± 2 3 mM Pb+Noise – 57 ± 4 3 mM Pb+Cd+Noise – 59 ± 4 Bone (mg/kg): Fem. 3 mM Pb – 287 ± 45Tib. 3 mM Pb – 216 ± 51 Fem. 3 mM Pb+Cd – 236 ± 24Tib. 3 mM Pb+Cd – 206 ± 23
13.	Jamesdaniel et al. (2018)	C57BL/6 mice	C: 6 Pb: 6 Pb+Noise: 6	4 weeks + 5 days acclimatized	PbAc	In drinking water 2 mM PbAc	28 days	BLL (µg/dL): Pb – 293 ± 67Pb +Noise – 319 ± 44
	B) *IN VITRO* STUDY							
14.	Liang et al. (2004)	Guinea pig cochlear cells	3-4 outer hair cells per concentration	Adult	PbAc	0.1, 1.0, 10, 100 µM baths	-	-

* blood Pb levels were not reported for treatment of 10 mg Pb.
PbAc – lead acetate; TEPb – tetraethyl lead; C – control; M – male; F – female; ip – intraperitoneal

Table 2. Outcomes and conclusions summarized for 14 toxicology Pb ototoxicity studies.

#	Study	Outcomes and conclusions	Other
	In Vivo Studies		
1.	Gozdzik-Zolnierkiewicz and Moszynski (1969)	•Temporal bone analysis was done with silver impregnation for 16 animals: Sensory cells in inner ear, spiral, and vestibular ganglion displayed no pathology. •Examine VIII nerve pathology was normal in five animals and lesions including demyelination and axonal degeneration were present in eleven. •Sudan black staining was carried out in 16 animals: four were normal; segmental demyelination and axonal degeneration observed in twelve.	•Eight animals died. •All remaining had systemic toxicity (weight loss and weakness) due to Pb. •Five surviving animals showed paralysis of limbs.
2.	Wilpizeski (1974)	•Pure tone detection thresholds trained shock avoidance showed hearing within normal ranges throughout the experiment (N = 2, treated for 10 and 21 weeks). •Temporal bone analyses and VIII nerve fiber study showed no damage to hair cells or demyelination (N = 3).	•Five died during treatment and two were sacrificed when near death. •Two developed arm and leg transitory paresis. •Severe weight loss was observed, however vomiting diarrhea and anorexia were not.
3.	Takahashi et al. (1984)	•Latency of N1 was significantly increased after day 2. •P2 latency was significantly increased over the control after day 3. •The amplitude between P1 and N1 increased on day 1, but was not significantly different on day 2 or 3. •After day 6, P1-N1 amplitude was again significantly different.	•Body weight decreased substantially due to Pb treatment initially. •At day 8, treatment weights were no longer significantly different from controls. •Hematocrit significantly decreased due to Pb treatment at day 6.
4.	Yamamura et al. (1984)	•No changes to pseudothreshold or maximum output voltages were observed between control and all three experimental groups. •Action potential latency of N1 was significantly longer in the highest exposure group than the control. •Highest exposure conditions required a greater sound intensity (dB) to illicit similar action potential maximum output voltage regressions in comparison to control.	•All experimental animals lost weight on average. •Three died in 10 mg group •Four died in 15 mg group •Fourteen died in 20 mg group
5.	Yamamura et al. (1987)	•No changes in cochlear microphonics, neither the maximum output voltage nor the pseudothreshold. •Input-output function of action potential was different in highest exposure – output voltage was reduced especially below 20 dB (indicating VIII nerve axonal impairment).	•One died in 10 mg group •Five died in 15 mg group •Eight died in 20 mg group
6.	Yamamura et al. (1989)	•High-dose Pb exposure caused dysfunction of the VIII nerve. •Whole nerve action potentials elevated across treatment lengths; control was lowest, with all treatments compared to control 2 wks was 10 dB higher, 4 wks was 20 dB higher, and 5 wks was 25 dB higher.	•Three died in 2 wk group •Twelve died in 4 wk group •Twelve died in 5 wk group
7.	Hotta et al. (1996)	•Pb treatment alone did not cause cochlear electrophysiological changes. •Potassium ion concentration in the scala media was not altered. •Pb + noise groups displayed a significant decrease in AP output voltage from both control and Pb groups. •Pb + noise CM output and intensity were significantly lowered due to Pb and noise. •Pb + noise EP latency was significantly longer than controls and the combined exposure groups showed a lowered mean absolute value of negative potential.	•6 died in 4-week group not exposed to noise. •14 died in 4-week group exposed to noise. •No animals showed hind limb paralysis.
8.	Nagymajtenyi et al. (1996)	•Electrocorticogram from auditory centers showed decreases in amplitudes that were not significant, however displayed trends with dose and time. •Increases in mean frequencies also trended with dose and time, but were only significantly different from control in the 320 mg/kg for 12 weeks group. •Decreases in auditory electrocorticography - index again trended with dose and treatment time and were significantly different from controls only at the 320 mg/kg for 12 weeks group.	•Relative weights of organs from treated rats did not differ significantly from controls.

(*Continued*)

Table 2. (Continued).

#	Study	Outcomes and conclusions	Other
9.	Tuncel et al. (2002)	•Acute hearing loss within two hours of exposure. •TEPb had a higher degree of toxicity to cochlea, though Pb content is equal to the PbAc solution. •Compound action potential thresholds were elevated in the Pb acetate group over controls 5-10 dB and significant from tested frequencies 4-40 kHz TEPb exposure significant at 20 and 24 kHz. •Cochlear microphonics isopotential curves were not significantly different.	•Animals were euthanized 120 minutes after baseline recordings. •No changes in cochlear microphonics or compound action potential at single injection doses of 20 mg/kg Pb acetate and 17.1 mg/kg TEPb. •Results at doses in the study were not seen after 60 minutes of treatment.
10.	Fazakas et al. (2005)	•Analysis of total electrocorticography in the auditory centers showed decreases in delta activity for the high Pb dose and Pb+Hg+Alcohol group. These changes were not shown as significant. •Changes in auditory cortical evoked potential latency and durations following acoustic stimulation of 1 Hz 40 dB was not pronounced (and not shown).	•Weight gain in the high Pb group was significantly lowered. •Liver/brain weight was significantly lower in the Pb-high group, Pb-high+Hg, and Pb+Hg+alcohol. Lung/brain weight was also significantly lower in the Pb-high group.
11.	Liu et al. (2011)	•Significantly increased latencies for all ABR waves I-V were observed after Pb treatment, especially in males. •Amplitudes, especially for waves I and II were reduced, and to a greater degree in males.	•No animal loss or measures of systemic toxicity were reported. •Latencies of waves I-V in the Pb+Cu group were significantly lower than the Pb group.
12.	Carlson et al. (2018)	•No cochlear outer hair cell loss was observed due to Pb. •Pb exposure did not cause significant changes in ABR or DPOAEs •No significant changes in ABR peak and latency were observed due to Pb treatment. •Potentiation due to Pb and noise exposures were not observed in ABR results or outer hair cell counts. •Pb and Cd together did not alter auditory results observed from the highest Pb treatment alone.	•One animal died due to unrelated causes (urologic syndrome). •Mild lesions in the kidney were observed in 91% of 3 mM Pb: karyomegaly in the S3 tubular epithelium.
13.	Jamesdaniel et al. (2018)	•Pb treatment induced shifts of 8-12 dB (this was significant at the click, 4, 16, 24, and 32 kHz). •Pb treatment significantly downregulated oxidative stress genes Sod1, Prdx4, and Idh1 in cochlear RNA •Pb and noise treated animals had threshold shifts of 10-25 dB significantly higher than shifts due to noise exposure alone at the click stimulus, 4 and 32 kHz.	•Normal weight gain was not altered.
	In vitro Study		
14.	Liang et al. (2004)	•Potassium current was reduced over time; this reduction was dose dependent. •Outward voltage-gated potassium relative current increased with increasing doses of Pb. •After washing Pb, these changes were not reversed. •Changes are small and are not indicative of causing hearing loss.	•Cells selected were middle to apical areas of the cochlea; cells sensitive to mid- and low-frequency sounds.

possible neural damage, following Pb exposure; however, these findings may have been confounded by systemic toxicity effects. Two studies noted no auditory effects (Fazakas, Lengyel, and Nagymajtényi 2005, Wilpizeski 1974), though these negative results could have resulted from very small sample size (i.e., Wilpizeski 1974), or the use of uncommon testing procedures (i.e., the auditory cortical evoked potentials and electrocorticography used by Fazakas, Lengyel, and Nagymajtényi 2005).

Studies displaying positive results for auditory dysfunction following high doses of Pb predominately used auditory brainstem response (ABR) and analysis of thresholds or waveforms. ABR can be performed in humans and rodents; five main waves are measured as neuronal signals pass from the cochlea to the auditory cortex in the brain. These main waves comprise an afferent pathway traveling sequentially through five major components of auditory processing: Eighth cranial nerve fibers beginning in the cochlea; eighth cranial nerve fiber upon entry to the Cochlear nucleus; action potentials exiting the cochlear nucleus and projecting to the superior Olivary complex; the signal in the Lateral lemniscus; and finally the Inferior colliculus within the midbrain of the brainstem (easily remembered as the underlined text shows ECOLI) (Jewett and Williston 1971; Picton et al. 1974). Following this succession of action potentials, the afferent signal is sent to the medial geniculate within the thalamus and further to the auditory cortex where processing occurs within the temporal lobe (Bartlett 2013). These last processing steps are essential for understanding and recognition of human speech.

Alterations in the amplitude or latency of these waves can be signals of pathology. Hearing thresholds, interpreted as waveforms present, but diminishing in amplitude as the stimulus presented quiets, are another measure of hearing ability. In humans, thresholds from 0 to 19 dB are considered within the normal hearing range. Thresholds of 20 dB or more demonstrate different degrees of HL; thresholds of 20–34 dB show mild HL, 35–49 dB show moderate HL, 50–64 moderately severe, 65–79 dB show severe, 80–94 dB show profound HL, and 95 dB and over show complete HL (Vos et al. 2015). The World Health Organization defines HL as thresholds at or above 25 dB in one or both ears for pure-tone single frequency audiometry (World Health Organization 1991). Mean auditory thresholds vary by species and stimuli (particularly the frequency or pitch), but are similar to humans (Zheng, Johnson, and Erway 1999).

Takahashi, Okamoto, and Saito (1984) found a significant increase in latency of N1 and a significant increase in P1-N1 amplitude after the sixth day of treatment. A significantly longer latency of N1 action potential was observed by Yamamura et al. (1984). Output voltage lowered under 20 dB in the highest Pb treatment group of Yamamura et al. (1987). Whole nerve action potentials were elevated to 25 dB (the authors did not specify baseline or final levels) after five weeks of treatment in results from Yamamura et al. (1989). Histopathology explored by Gozdzik-Zolnierkiewicz and Moszynski (1969) showed axonal degradation and demyelination in the vestibulocochlear nerve (VIII cranial nerve), but no pathology in inner ear cells or in the spiral ganglion.

The study that used a terminal procedure explored the effects of Pb, both Pb acetate (PbAc) and tetraethyl Pb on cochlear microphonic and compound action potentials thresholds. In guinea pigs, auditory nerve compound action potentials were disrupted due to administration of Pb acetate and increased thresholds by 5–10 dB at high and mid frequencies (Tuncel, Clerici, and Jones 2002). Threshold shifts in the tetraethyl Pb group were significantly greater than controls.

Low Pb dose studies

The four studies that administered lower doses of Pb employed different measures of auditory function and generally demonstrated ototoxicity. Nagymajtenyi, Schulz, and Desi (1996) used electrocorticography, but in contrast to the study with negative results in a high dose of Pb, found a significant decrease in electrocorticography index in the auditory centers, and a significant increase in mean frequencies for the study's highest and longest treatment group. Liu et al. (2011) found changes in ABR wave I-V latencies in rats exposed to Pb acetate via gavage. Jamesdaniel et al. (2018) used ABR in mice to determine significant 8–12 dB shifts from baseline threshold at 4, 16, 24, and 32 kHz. In contrast, Carlson, Schacht, and Neitzel (2018) did not find significant changes in

threshold shifts or distortion product otoacoustic emissions (DPOAEs) after Pb treatment, and no outer hair cell loss in the cochlea.

In vitro *studies*

The majority of animal studies included in this review are laboratory *in vivo* studies. However, one *in vitro* study of cochlear outer hair cells from adult guinea pigs is summarized in Tables 1 and 2. This study showed alterations of neural signaling necessary for auditory processing following exposure to Pb. PbAc decreased outward potassium currents in adult pigmented guinea pigs outer hair cell explants (Liang et al. 2004).

Further, *in vitro* studies on neurological tissues indicate possible mechanisms for Pb toxicity in the auditory system, though the following studies were not performed with auditory tissues. Nerve terminals from rat brains receiving Pb treatment impacted the crucial proton gradient of synaptic vesicles and decreased glutamate accumulation inside these synaptic vesicles (Borisova et al. 2011); this is related to auditory processing, as glutamate is the primary neurotransmitter used in ascending auditory neurons (Raphael and Altschuler 2003).

Mixed exposures

While metals are often tested alone under controlled laboratory conditions, environmental and occupational exposures usually contain a mixture of different types of metals and different combinations of metals. Results from studies which manipulated the Pb-exposures via the addition of another agent, either protective or harmful, have been summarized here.

In combinations with other agents, several studies still showed ototoxic effects for Pb; for example, acute low Pb doses of PbAc caused delayed auditory wave latencies in rats, which were alleviated through doses of copper (Liu et al. 2011). Phenyl-tert-butylnitrone administration alleviated threshold shifts from tetraethyl Pb to some degree (Tuncel, Clerici, and Jones 2002). Other studies found negative results. Fazakas, Lengyel, and Nagymajtényi (2005) suggested that alcohol administration counteracted the effects due to Pb, but changes in electrocorticography in the auditory center due to a combination of Pb, mercury, and alcohol administration were not significantly different from a control group.

In vivo studies are necessary to understand the combination of physical and chemical hazards that have been explored only in two recent studies. Carlson, Schacht, and Neitzel (2018) did not observe any significant changes in thresholds from combinations of Pb and cadmium; Pb, cadmium, and noise; or Pb and noise. Jamesdaniel et al. (2018) observed greater threshold shifts to animals exposed to both Pb and noise compared to mice exposed to Pb alone, suggesting potentiation.

Human studies

We reviewed 24 epidemiological studies. The methodological features of these studies are summarized in Table 3, and study findings are summarized in Table 4. In reviewing these papers, we identified four areas of uncertainty and weakness in this literature: 1) hearing examination and HL quantification; 2) Pb exposure assessment; 3) noise exposure assessment; and 4) participant characteristics. We describe each of these in areas in detail in the sections below.

Hearing examination and HL quantification

The largest area of concern and variation with epidemiologic study methodology was the varying quality and type of hearing examinations. Good hearing requires a functional outer, middle, and inner ear, and unimpeded transmission of neural signals from the vestibulocochlear nerve, through the brainstem, to the primary auditory cortex in the cerebral cortex of the brain. An important need for both toxicological and epidemiological studies is to examine the location and mechanism of hearing damage due to Pb exposure, and its interaction with noise. For example, while noise-induced damage typically manifests as cochlear hair cell death in the basal region, the toxicological studies suggest damage due to Pb takes place in the neural processing networks. Without this knowledge, the optimal measure (or measures) of hearing is unclear.

The 24 included human studies used a range of hearing tests, including DPOAEs, pure-tone audiometry, and brainstem auditory evoked response (BAER), each of which measures different aspects of the hearing system. DPOAEs, used in two human studies, are best used to

assess cochlear outer hair cell function. Audiometry, used in 15 studies summarized for this review, identifies the lowest level of subjectively detectable sound. Finally, BAER, also called brainstem auditory evoked potentials (BAEP), used in 11 studies, measures neuronal transmission of action potentials to the auditory center of the brain. As Pb is a known neurotoxicant, further exploration into modification of auditory neural processing through BAER is likely worth investigation in Pb ototoxicity studies. Past studies have found that correlations between pure-tone audiometry and DPOAEs may be poor (Engdahl, Tambs, and Hoffman 2013). Determining the best auditory function measures for epidemiological analysis will require a more complete understanding of mechanisms surrounding Pb ototoxicity.

Regardless of the test used, a quiet environment is necessary to ensure accurate hearing measurements. Four studies described the environment where tests occurred; background noise levels during these tests ranged from a "quiet area" (Counter and Buchanan 2002) to a sound-treated chamber (Park et al. 2010). The background levels of 30 (Hwang et al. 2009) and 50 dB (Wu et al. 2000) in two human studies are troubling, as normal thresholds are in the range of 0–20 dB. Prior to testing, tympanometry and visual inspection of the tympanic membrane to rule out ear infections or obstructions are needed. However, these tests were only described by one (Farahat et al. 1997) of the 24 studies.

Temporary threshold shifts due to noise exposure are another important consideration that could substantially alter the results of hearing tests and attenuate potential relationships between Pb, noise, and HL. Only two of the 24 human studies gave details on the timing of tests after the last exposure to high noise: 14 (Wu et al. 2000) and 16 (Chuang et al. 2007) hours of quiet, respectively.

Finally, due to the resilient nature of the hearing system (Pepler et al. 2014), it may be essential to rely on multiple tests to determine deficits in hearing function. To address this limitation, we recommend the use of BAER to evaluate auditory neural processing, in combination with pure-tone audiometry to evaluate the perception of sound.

Pb exposure analysis

Biological Pb levels were measured in all of the 24 studies. Blood lead levels (BLLs) were the most frequently used assessment technique reported by 22 studies. Other techniques included measurements of airborne concentrations of Pb (used in addition to biological Pb), measurement of blood Pb in fingernails, and measurements of bone Pb. While blood lead levels (BLLs) may be representative of recent exposures, they do not account for the accumulation of Pb in the bones following chronic exposure. Blood Pb has a half-life of about 30 days (Roberts et al. 2001), depending on initial levels, but also represents an equilibrium of the body burden carried in the bone. Bone Pb has a half-life over decades or longer (Wilker et al. 2011). Two epidemiological studies used time-weighted averaging approaches to address the critical issues of exposure timing and intensity (Bleecker et al. 2003, Hirata and Kosaka 1993). One study measured the duration of exposure and Pb concentrations in ambient air (Hirata and Kosaka 1993), and both studies investigated the gold standard: individual monitoring of past BLLs from workers' five-year history. Park et al. (2010) took advantage of a recent development in measuring cumulative Pb exposure and used X-ray fluorescence to quantify Pb in bone. Use of this approach should increase the ability to accurately estimate metals exposure over long periods of time where BLLs are not especially meaningful.

Noise exposure assessment

Noise exposure is a major concern in studies of workers, since noise is a nearly ubiquitous occupational exposure. In some workplaces, Pb and noise exposure may co-occur, requiring an even more thoughtful exposure assessment to evaluate the exposures concurrently. Five (Bleecker et al. 2003, Chuang et al. 2007, Farahat et al. 1997, Hwang et al. 2009, Wu et al. 2000) of the 24 included occupational studies suggested HL in workers exposed to both noise and metals greater than expected for the amount of noise exposure alone. However, none of these studies provided sufficient information on the levels of occupational noise to which workers were exposed. Only one study investigated a link between noise exposure and heavy metals exposures, in this case among mining occupations (Saunders et al.

Table 3. Methodological features of 24 epidemiological Pb ototoxicity studies.

#	First Author (Year)	Type	N	Sex (F/M)	Mean Age	Pb Exposure Setting	Place	Hearing Test	Pb Measure	Noise	Metals
1	Baloh et al. (1979)	Cross-sectional	69 (64 aud.); 35 (31 aud.) controls	-	43 (Spivey et al. 1979) (sd = 11)	Secondary Pb smelter – refining, and recasting Pb from auto batteries; controls from aluminum processing facility (Spivey et al. 1979)	US – southern CA	Pure-tone conduction; impedance studies; speech recog.	Current BLL 61 (13) µg/100 mL; Longitudinal BLL 60 (14) µg/100 mL	No	As
2	Spivey et al. (1980)	Pro-spective	69 (64 aud.); 35 (31 aud.) controls	-	-	Secondary Pb smelter; controls from aluminum processing facility	US – southern CA	Pure tone conduction; impedance studies; speech recog.	Follow-up BLL 66 (13) µg/dL;	No	No
3	Holdstein et al. (1986)	Cross-sectional	57; Older: 16 exposed and 20 controls	Older exposed 10 F/6 M	Young (8–17); Older (18–56)	Ingestion of Pb-contaminated food source for 1–2 years, study was conducted one year later	-	Pure-tone thresholds and BAER	Prior BLL were averaged with new levels. Means of older: 31.2 µg/dL, prior 43.4 µg/dL	No	No
4	Lille et al. (1988)	Cross-sectional	13 Pb workers; 20 controls	3 F/10 M	37 (sd = 10)	Occupationally exposed to inorganic Pb compounds	-	BAEP	Mean BLL 100 µg/dL (range 27–240)	No	No
5	Araki et al. (1992)	Cross-sectional	22 workers; 14 controls	22 M	Median 48 (range 32–58)	Gun metal foundry workers	-	Auditory event-related potential	Median BLL 30 µg/dL (range 12–59) (UPb values not reported)	No	Zn, Cu
6	Discalzi et al. (1992)	Cross-sectional	49 workers; 49 controls (ASM)	12 F/37 M	34 (sd = 11)	31 from Pb battery factories; 7 from the ceramic industry; 4 Pb contaminated wine; 7 misc. occupational exposures	Europe	BAEP	Mean BLL 55 (16) µg/dL; mean 3-year BLL average 54 (16) µg/dL	No	No
7	Discalzi et al. (1993)	Cross-sectional	22 workers; 22 controls	5 F/17 M	35 (sd = 12)	Pb storage battery factory workers	Italy	BAER	BLL 48 (sd = 11) µg/dL	No	No
8	Hirata and Kosaka (1993)	Cross-sectional	41 Pb workers/39 controls	All M	19 to 58	Pb-exposed workers from 4 factories and controls from nylon factory.	Japan	ABR	Current BLL and TWA from the past 5 years.	No	No
9	Murata et al. (1993)	Cross-sectional	22 workers; 22 controls	22 M	Range 32–59	Gun metal foundry worker	-	BAEP	Mean BLL 39 µg/dL (range 16–64 µg/dL)	No	No
10	Murata et al. (1995)	Cross-sectional	36 Pb workers/15 textile controls	All F	21 to 35	Glass workers (7.8 average years in factory)	China	BAEP	Mean BLL 56 µg/dL (range 26–79); Air concentrations 0.4–1.2 mg/m^3	No	No
11	Farahat et al. (1997)	Cross-sectional	45 workers/45 controls	–	20 to 40	Printing facility and controls from textile factory	Egypt	Pure-tone thresholds	BLL (worker mean 37 µg/dL; control mean 12 µg/dL)	40–50 dB	No
12	Forst, Freels, and Persky (1997)	Cross-sectional	183 workers	12 F/171 M	19 to 65	Private business. 70% white, 22% African American, 8% Hispanic	US	Pure-tone thresholds	BLL 1–8 µg/dL (5 µg/dL 50th percentile)	No	No

(*Continued*)

Table 3. (Continued).

#	First Author (Year)	Type	N	Sex (F/M)	Mean Age	Pb Exposure Setting	Place	Hearing Test	Pb Measure	Noise	Metals
13	Fujimura et al. (1998)	Cross-sectional	2 workers/ 42 controls	2 M	50 & 57	Pb smelting	-	BAEP	Mean BLL 104 & 79 µg/dL (followed for 12 and 15 months)	No	No
14	Buchanan et al. (1999)	Cross-sectional	5 adults	1 F/4 M	Range: (17–51)	Ceramic production and Pb glazing from Pb-acid batteries	Village of LaVictoria in Ecuador	DPOAE, ABR, & Pure-tone threshold	Mean BLL 41 µg/dL (range 19–56)	No	No
15	Wu et al. (2000)	Cross-sectional	220 workers	102 F/ 118 M	37 (sd = 10)	Workers at two Pb-battery factories	Taiwan	Pure-tone thresholds	BLL as short-term and long-term value calculated from ambient Pb concentrations and years working at plant	LEQ for each individual	No
16	Counter and Buchanan (2002)	Cross-sectional	30	15 F/ 15 M	Median age 35 (17–55)	Pb glazing workers	Ecuador	Pure-tone thresholds. BAERs on 12 participants	BLL 45 (20 = sd) µg/dL. Range of 11–80 µg/dL.	Reported in survey	No
17	Yokoyama et al. (2002)	Cross-sectional	29	All F	27 (22–29)	Pb glass factory workers (employed for 3–17 years, mean 8)	Beijing, China	BAEP	Mean BLL 56 µg/dL (range: 26–79)	No	No
18	Bleecker et al. (2003)	Cross-sectional	359	All M	41 (sd = 9)	Pb smelting workers. French and English speaking.	-	BAER	BLL, TWA from past 5 years, and integrated blood Pb measure – overtime working at plant.	No	No
19	Chuang et al. (2007)	Case-Control	121 HL/173 controls	All M	Cases 44 (sd = 9)/ Controls 40 (sd = 9)	Outpatients and occupational health examination workers with HL. Controls patients with no HL	Taiwan	Pure-tone thresholds	BLL geometric mean (11 µg/dL – cases/4 µg/dL – controls)	Reported yes/no, if yes years of duration	Blood Mn, As, Se
20	Hwang et al. (2009)	Cross-sectional	412 total, 395 factory/ 17 admin.	1 F/ 411 M	36 (7 = sd)	Steel workers	Taiwan	Pure-tone thresholds	BLL Grouped under 4 µg/dL, 4–7 µg/dL, and over 7 µg/dL	Groups <80, 80–85, and >85 dB	Mn, Cu, Zn, As, Cd
21	Park et al. (2010)	Cohort	448	All M	65 (7 = sd)	Normative aging study-veterans	Eastern MA	Pure-tone thresholds	Bone (tibia and patella) Pb	Job title	No
22	Choi et al. (2012)	Cohort	3698	1969 F/ 1729 M	42 (0.3 = se)	NHANES – representative civilian US population	US	Pure-tone thresholds	BLL Geometric mean 1.54 (95% CI: 1.49, 1.60) µg/dL	Firearms, recreational, and job title	Cd
23	Saunders et al. (2013)	Cross-sectional	59	10 F/ 49 M	Range: 9–78	Artisanal gold miners	Nicaragua	Pure-tone thresholds, DPOAE	Finger nail (some toe) clippings. Median 3.93 µg/g	Reported Low/High	Hg, As, Mn, Al
24	Huh, Choi, and Moon (2016)	Cross-sectional	7596	4843 F/ 4115 M*	Range: 10–87	2010–2013 National Health Survey	Korea	Pure-tone thresholds	BLL – weighted geometric mean 2.08 µg/dL 95% CI (2.05, 2.11)	Survey: Loud, occ., firearms	No

Table Abbreviations: ABR – auditory brainstem response (very similar to BAER); admin. – administration workers; ASM – age and sex matched; BAEP – brainstem auditory evoked potentials; BAER – brainstem auditory evoked response; BLL – blood lead levels; dB – decibels; DPOAE – distortion product otoacoustic emissions; F – female; Hz – hertz; LEQ – Equivalent Continuous Sound Level (measured in dB); Loud – loud noise group; M – male; MA – Massachusetts; µg/dL – micrograms Pb per deciliter blood; Occ. – occupational; Pb – lead; sd – standard deviation; se – standard error; TWA – time weighted average. *Obtained from downloading available datafile.

Table 4. Summary of Pb ototoxicity findings from twenty-four human study.

#	Paper	Summary of findings
1.	Baloh et al. (1979)	•No significant differences between control and Pb group (baseline to study below).
2.	Spivey et al. (1980)	•No significant changes from previous audiometric results (above) (Baloh et al. 1979) after 12 to 18 months of BLL monitoring and follow-up testing.
3.	Holdstein et al. (1986)	•Normal hearing threshold levels. •Significant difference between exposed and control IPL I-III with stimulus delivered at 10/s and 55/s. •A negative correlation between BLL and IPL for wave III-V was significant.
4.	Lille et al. (1988)	•All BAEP results were found to be within normal ranges aside from one patient. •One Pb-exposed alcoholic participant was found to have an increased I-V interpeak latency of 4.7 ms.
5.	Araki et al. (1992)	•Auditory ERP P300 component was prolonged significantly in Pb workers compared to control. •Auditory ERP P300 latency in Pb workers was correlated (Pearson's product moment correlation coefficient) significantly with BLL, UPb (not shown). •Controls had significantly lower BLL, plasma Zn, and plasma Cu.
6.	Discalzi et al. (1992)	•No significant correlation for linear regression was found between 3-year BLL, BLL, or Pb exposure duration and BAEP latencies (I, III, and V). •All BAEP latencies and interpeak latency differences were significantly different in Pb workers compared to controls. •When comparing 21 Pb working participants with an average 3-year BLL above 50 µg/dL to 28 participants below 50 µg/dL, a significantly longer I-V interpeak latency was observed.
7.	Discalzi et al. (1993)	•Greater I-V mean IPL between controls and workers. •Greater I-V mean IPL in workers with Pb levels over 50 µg/dL and those under 50 µg/dL.
8.	Hirata and Kosaka (1993)	•Latency of peak III-V was increased significantly in the Pb-exposed. •Latencies for individual peaks I and III were significantly longer in the unexposed workers.
9.	Murata et al. (1993)	•All BAEP latencies (I, III, and V) were not significantly different between Pb workers and controls. •BAEP latency I-V and V were significantly correlated with packed cell volume in 20 workers. •Auditory ERP P300 latencies were significantly longer in Pb workers compared to controls. •Auditory ERP P300 latencies was significantly correlated (simple correlation coefficient) in 22 workers with BLL, urinary Pb, years of employment, urinary Zn, and age.
10.	Murata et al. (1995)	•No significant differences in BAEP latencies (I, I-III, or I-V) were found between Pb workers and controls.
11.	Farahat et al. (1997)	•8 kHz was significantly different between workers with BLL < 30 µg/dL and those ≥ 30 µg/dL. •Threshold at 2, 4, and 8 kHz were significantly different in workers and controls. •A significant positive correlation was observed at 8 kHz between BLL and threshold.
12.	Forst et al. (1997)	•High frequency HL was evident in cohort. •Significant Spearman correlation with threshold and BLL at 4 kHz. •Trend tests were not significant.
13.	Fujimura et al. (1998)	•BAEP latency V was significantly different than expected values for Pb smelter #1. •Auditory ERP P300 latency was significantly different from control for Pb smelter #1.
14.	Buchanan et al. (1999)	•Two of five participants reported noise exposure histories. One participant with history of noise exposure had thresholds of 110 dB at 6 kHz in one ear. •Range of mean hearing thresholds were 9.5 dB at 2 kHz to 32 dB at 6 kHz. •At high frequencies mean DPOAE amplitudes show lowered levels consistent with noise exposure. •No significant associations between DPOAE and BLLs were observed.
15.	Wu et al. (2000)	•Age was correlated with HL. •Long-term Pb exposure metric was significantly associated with HL, though correlated with age. •Increasing thresholds were noted when groups were stratified by BLL (25-40, 41-60, and over 60 µg/dL); however the lowest group (below 25 µg/dL) did not fit this relationship.

(*Continued*)

Table 4. (Continued).

#	Paper	Summary of findings
16.	Counter and Buchanan (2002)	•More HL in men than women. •Four case profiles with high Pb levels described. •No significant relationship between BLL and thresholds. •Those with hearing loss displayed longer (non-significant) absolute wave latencies, but normal IPL.
17.	Yokoyama et al. (2002)	•No significant differences between Pb workers and controls were observed in BAEP latencies (I, I-III, and III-V). •BAEP latencies were not found to correlate with BLLs.
18.	Bleecker et al. (2003)	•Peak I & V latency significantly correlated with Integrated Pb exposure, BLL and TWA. •Peak III latency correlated significantly only with TWA, and integrated Pb exposure. •When stratifying by BAEP pathology, the group with longest wave I latency and I-V IPL had significantly higher BLL and TWA than the group with normal wave I latency and normal I-V IPL.
19.	Chuang et al. (2007)	•Age was associated with HL. •Increasing selenium levels were protective against HL. •An increase of 0.1 µg/dL of log-transformed BLL significantly associated with a 7 dB increase in HL.
20.	Hwang et al. (2009)	•Higher BLLs increased risk of HL in high frequencies of 3, 4, 6, and 8 kHz. •Higher BLLs were significantly correlated with hearing loss at 0.5, 2, 3, 4, 6, and 8 kHz as well as the average noise and maximum noise levels. •Levels above 7 µg/dL were significantly associated with hearing thresholds above 25 dB (odds ratios 3.06-6.26) in logistic models adjusting for noise and age.
21.	Park et al. (2010)	•Odds of HL significantly increased with bone (patella) Pb levels. •A positive interaction was found between bone (tibia) Pb levels and time in the linear mixed effects model showing a faster elevation of thresholds with increasing levels of Pb. •Effect modification analysis showed non-linear dose-response for threshold changes and low, medium, and high occupational noise.
22.	Choi et al. (2012)	•Age-adjusted BLLs were higher in participants who were older, less-educated, smokers, those with high occupational noise exposures, those with a BMI over 30, and those without diabetes. •Participants with HL had significantly higher age-adjusted geometric mean BLL (0.46 to 0.40 µg/dL). •BLL were significantly correlated with blood cadmium levels. •Highest BLL quintiles had 18.6% (95% CI: 7.4, 31.1%) higher average thresholds than those in the lowest quintiles. •Models showed the average thresholds trended across all quintiles significantly with BLL before and after adjusting for occupational and recreational noise exposures. •In logistic models (using a yes or no for hearing loss defined as an average of thresholds at four frequencies over 25 dB) BLL was found as a significant predictor of HL, however it was no longer significant when noise exposure was adjusted for.
23.	Saunders et al. (2013)	•No meaningful significant relationships found in the group as a whole after Bonferroni corrections. •DPOAE at 3 kHz, 4 kHz, and the mean of all DPOAE frequencies were significantly correlated with BLL before Bonferroni corrections. •Three case reports of workers with high metals exposure and hearing loss.
24.	Huh et al. (2016)	•Risk of hearing loss (defined as pure-tone average at or over 25 dB) increased for participants with BLLs above the mean (OR = 1.14, 95% CI: 0.42, 3.13). •Increasing levels of Pb significantly trended with increasing hearing loss in a fully adjusted model – controlling for age, sex, smoking status, monthly income, education levels, body mass index, occupational noise exposure, loud noise exposure, firearm noise exposure, hypertension, and diabetes mellitus. A 43% higher odds of hearing loss (95% CI: 1.03-2.00) was shown in this model for every 1 µg/dL increase in BLL. •The highest quintile of BLL (2.9-26.5 µg/dL) showed significant increased risk for hearing loss across two other models adjusting for fewer variables. However the trend across quintiles in these other models was not significant.

Table Abbreviations: BLL – blood lead levels; CI – confidence interval; HL – hearing loss; IPL – interpeak latency; OR – odds ratio; Pb – lead; TWA – time weighted average.

2013). The findings were inconclusive due to confounding issues of poor health as well as the large number of statistical analyses performed on multiple metals.

Quantification of environmental or leisure noise exposure is also critical. Of the 24 epidemiological studies included, two (Choi et al. 2012, Huh, Choi, and Moon 2016) included some adjustments and exploration into sources of noise that were non-occupational. Firearms create unique blast noise exposure, which is a threat to the entirety of the hearing system. There is also potential of additional Pb exposure from the use of firearms. Moreover, the use of guns is often correlated with high occupational noise exposure (Agrawal, Niparko, and Dobie 2010). Determination of leisure activities and community settings where loud noise exposures occur is essential. Communities feature multiple sources of noise exposure – e.g. multiple modes of transportation, recreational activities, etc. (Neitzel et al. 2012) – and all of these sources must be assessed to comprehensively evaluate the risk of HL with or without the presence of Pb.

Participant characteristics

Demographic information for participants was difficult to evaluate in a number of the human studies we reviewed. Every study listed participant age ranges and accounted for increased HL with age; however, this was often confounded by Pb exposure metrics that also increased with age or duration of employment. Given the correlation between these factors, more thoughtful analysis is needed. As HL levels have been found to vary by race (Hoffman et al. 2017, Sun et al. 2014) and income level (Chou et al. 2015), detailed information on participants characteristics is necessary for a clear understanding of possible confounders. Choi et al. (2012) had a great deal of information on their participants and detailed hearing outcomes as significantly different for non-Hispanic whites and non-Hispanic blacks; this study also found borderline significant differences between BLL and threshold levels for Mexican-Americans and non-Hispanic whites.

Potential confounders

In addition to these four sources of uncertainty in the identified studies described above, there are two major areas of potential confounding (protective factors and exposures to mixtures of ototoxicants) that need to be addressed more systematically. These are described below.

Protective factors

Factors which could improve the health of the hearing system and/or lower levels of Pb in the system must also be accounted for in human studies. High dietary intakes of iron and zinc have been shown to decrease the absorption of Pb (Goyer 1997). This may modify the effects of exposure to Pb in the ambient environment. Chuang et al. (2007) examined possible protective levels of selenium and found them significant. Araki et al. (1992) and Hwang et al. (2009) both accounted for levels of zinc and copper in the blood, which are essential metals and can limit uptake of more non-essential metal uptake by the body. To account for these dietary factors, a dietary survey must be used, as levels of zinc and iron in the blood are generally held in strict homeostatis and are not reflective of intake. Hearing protection offers workers and other exposed individuals protection against high noise, and can dramatically modify ambient exposure levels. None of the studies in this review accounted for hearing protection, even though this can attenuate exposures over 30 dB (Sayler et al. 2018). Therefore, assessment of exposure attenuation provided by hearing protectors must be included in epidemiological studies.

Exposure to mixtures of ototoxicants. The number of known ototoxicants has increased in recent years; a partial list of the most relevant exposures that should be considered includes: smoke, drugs, solvents, and other potential metals. Adequate control of these measures in epidemiological studies requires large data sets and extensive participant information. Choi et al. (2012) used a large data set and included measures of Pb, cadmium, occupationally related noise exposures, and non-occupational noise exposures. Smoking and environmental tobacco smoke are important exposures, although self-reported exposures have been shown to be unreliable (George et al. 2006). Both active and passive exposures to smoke should be assessed, as literature has shown a link between

secondhand smoke and HL in adolescents (Lalwani 2011). One study we summarized had detailed questionnaire data on participant smoking and was able to use pack-years in linear regression models (Park et al. 2010).

Powerful pharmaceutical agents, including cisplatin and aminoglycoside antibiotics, can cause damage to the auditory system (Schacht, Talaska, and Rybak 2012). Other drugs, including the commonly used pain medications aspirin and ibuprofen, as well as the angiotensin-converting enzyme (ACE) inhibitor Ramipril, can cause tinnitus, temporary HL, or permanent HL (Bisht and Bist 2011). One of the studies from our review documented participant ototoxic drugs by self-report (Choi et al. 2012) and one study discussed likely exposures to ototoxic medications for their participants (Saunders et al. 2013).

Solvents, including toluene, styrene, xylene, carbon disulfide, and trichloroethylene, exhibit ototoxic properties (Hughes and Hunting 2013). Styrene, for example, exacerbates HL in the presence of concurrent noise exposures (Mäkitie et al. 2003), and jet fuel (a mixture of chemicals) can cause HL (Fechter and Pouyatos 2011) and has potentiated HL from noise (Fechter et al. 2012, Guthrie et al. 2015, 2014). None of these 24 human studies assessed possible solvent exposure.

While properties of lead ototoxicity are unclear at this time, ototoxic properties of other metals have been reported and are less researched. The degree that metals other than Pb – e.g., mercury, cadmium, and arsenic – may influence hearing outcomes needs to be evaluated in epidemiological studies. Dietary reports and occupational histories, which were not assessed by any of these studies, are needed to account for comprehensive exposure assessment of essential and non-essential metal exposures. Of the five studies considering other metal exposures (both toxic and essential), all of them used biological markers. Hair levels of arsenic were recorded (Baloh et al. 1979), metal levels in fingernails was used in one study (Saunders et al. 2013), plasma zinc and copper were used in another study (Araki et al. 1992), and all others measured levels of arsenic, manganese, selenium, and cadmium in whole blood (Baloh et al. 1979, Choi et al. 2012, Chuang et al. 2007).

Future opportunities

Accurately examining potential modification and confounding of Pb ototoxicity by noise exposures will allow for more protective health policies and procedures. Workers exposed to Pb have been shown to have higher levels of oxidative stress (Khan et al. 2008). These levels may have negative effects on their hearing ability. Encouragingly, changes to neurobehavioral performance have been shown to be reversible in Pb workers when exposures were reduced (Chuang, Chao, and Tsai 2005). This may also be the case for negative effects of Pb on hearing.

Relevant health policy changes have recently occurred for environmental Pb exposures. In 2012, the CDC lowered the community action limit of Pb in the blood to 5 μg/dL (CDC (US Centers for Disease Control and Prevention) 2012). Conversely, the Occupational Safety and Health Administration has not updated its BLL standards since 1978 (40 Fed. Reg. 52952 [1978]). This agency requires workers whose BLL are at or above a 40 μg/dL limit to receive medical examinations. The medical provisions of 29 CFR § 1910.1025 could be amended to require a hearing examination to assess the possible influence of Pb on auditory outcomes. This modification could assist in investigating the links of Pb and HL. Improving the safety of workers exposed to lead in the US can be improved as NIOSH has recently lowered their definition of an elevated BLL in adults to 5 μg/dL (NIOSH (US National Institute for Occupational Safety and Health) 2018).

Publication of all studies exploring the ototoxic nature of Pb is needed, whether outcomes are positive or negative. While the results of the available literature suggest some consensus of ototoxic findings, it is worth considering that these results may have been influenced by publication bias and the relative absence of published studies with negative results (Song, Loke, and Hooper 2014).

Conclusions

As our understanding of the impacts of ototoxic exposures on the auditory system expands, and we better understand complex interactions in dynamic biological systems, it is important to evaluate the role that both chemical and physical agents play in the

hearing system and how they interact together. Overall, the 38 studies included in this review did not show a clear relationship between Pb exposure and auditory health outcomes. At environmentally and occupationally relevant doses, the epidemiological evidence from 24 human studies seems to provide stronger support at this time than data from the 14 animal studies, many of which focused on much higher exposures. Collectively, the majority of these 38 studies suggest a possible relationship between Pb and HL, and five studies hint at an interaction with noise-related HL pathology. However, it is important to note that the majority of the human studies included in this article are cross-sectional studies, which contributes to the weakness in establishing a direct link between lead exposures and hearing loss in epidemiological studies. Future studies should incorporate longitudinal study designs where possible and address the four areas of uncertainty identified here: hearing examination and HL quantification; Pb exposure assessment; noise exposure assessment; and participant characteristics. Additionally, studies must better address potential confounding from protective factors and exposures to mixtures of ototoxicants. Further studies on HL and Pb exposure are essential to developing a better comprehension of the Pb exposure levels of concern and the physiological processes that are most vulnerable to the possibility of Pb ototoxicity.

Acknowledgments

The authors wish to thank Marie O'Neil for her support and feedback regarding this review, and Stephanie Sayler for assistance in formatting and editing.

Disclosure

The authors declare no conflicts of interest related to this research.

Funding

This work was supported by aUniversity of Michigan Rackham Merit Fellowship and a Environmental Toxicology and Epidemiology Program fellowship funded by the National Institute of Environmental Health Sciences [T32 ES007062].

References

Agrawal, Y., J. K. Niparko, and R. A. Dobie. 2010. Estimating the effect of occupational noise exposure on hearing thresholds: The importance of adjusting for confounding variables. *Ear. Hear.* 31:234–37. doi:10.1097/AUD.0b013e3181c6b9fd.

Anniko, M., and L. Sarkady. 1978. Cochlear pathology following exposure to mercury. *Acta Otolaryngol.* 85:213–24. doi:10.3109/00016487809121443.

Araki, S., H. Sato, K. Yokoyama, and K. Murata. 2000. Subclinical neurophysiological effects of lead: A review on peripheral, central, and autonomic nervous system effects in lead workers. *Am. J. Ind. Med.* 37:193–204. doi:10.1002/(ISSN)1097-0274.

Araki, S., K. Murata, K. Yokoyama, and E. Uchida. 1992. Auditory event-related potential (P300) in relation to peripheral nerve conduction in workers exposed to lead, zinc, and copper: Effects of lead on cognitive function and central nervous system. *Am. J. Ind. Med.* 21:539–47. doi:10.1002/(ISSN)1097-0274.

Baloh, R. W., G. H. Spivey, C. P. Brown, D. Morgan, D. S. Campion, B. L. Browdy, J. L. Valentine, H. C. Gonick, F. J. J. Massey, and B. D. Culver. 1979. Subclinical effects of chronic increased lead absorption – A prospective study. II. Results of baseline neurologic testing. *J. Occup. Med.* 21:490–96.

Bartlett, E. L. 2013. The organization and physiology of the auditory thalamus and its role in processing acoustic features important for speech perception. *Brain. Lang.* 126:29–48. doi:10.1016/j.bandl.2013.03.003.

Bisht, M., and S. S. Bist. 2011. Ototoxicity: The hidden menace. *Indian. J. Otolaryngol Head Neck Surg.* 63:255–59. doi:10.1007/s12070-011-0151-8.

Bleecker, M. L., D. P. Ford, K. N. Lindgren, K. Scheetz, and M. J. Tiburzi. 2003. Association of chronic and current measures of lead exposure with different components of brainstem auditory evoked potentials. *NeuroToxicology* 24:625–31. doi:10.1016/S0161-813X(03)00045-7.

Borisova, T., N. Krisanova, R. Sivko, L. Kasatkina, A. Borysov, S. Griffin, and M. Wireman. 2011. Presynaptic malfunction: The neurotoxic effects of cadmium and lead on the proton gradient of synaptic vesicles and glutamate transport. *Neurochem. Int.* 59:272–79. doi:10.1016/j.neuint.2011.05.010.

Buchanan, L. H., S. A. Counter, and F. Ortega. 2011. Environmental lead exposure and otoacoustic emissions in Andean children. *J. Toxicol. Environ. Health., Part A* 74:1280–93. doi:10.1080/15287394.2011.587106.

Buchanan, L. H., S. A. Counter, F. Ortega, and G. Laurell. 1999. Distortion product oto-acoustic emissions in Andean children and adults with chronic lead intoxication. *Acta Otolaryngol. (Stockholm).* 119:652–58. doi:10.1080/00016489950180586.

Campo, P., T. C. Morata, and O. Hong. 2013. Chemical exposure and hearing loss. *Disease-A-Month* 59:119–38. doi:10.1016/j.disamonth.2013.01.003.

Carlson, K., J. Schacht, and R. L. Neitzel. 2018. Assessing ototoxicity due to chronic lead and cadmium intake with

and without noise exposure in the mature mouse. *J. Toxicol. Environ. Health., Part A* 81:1041–57. doi:10.1080/15287394.2018.1521320.

Cary, R., S. Clarke, and J. Delic. 1997. Effects of combined exposure to noise and toxic substances – Critical review of the literature. *Ann. Occup. Hyg.* 41:455–65. doi:10.1016/S0003-4878(97)00006-9.

CDC (US Centers for Disease Control and Prevention). 2012. Response to advisory committee on childhood lead poisoning prevention recommendations in "Low level lead exposure harms children: A renewed call of primary prevention". http://www.cdc.gov/nceh/lead/ACCLPP/CDC_Response_Lead_Exposure_Recs.pdf.

Choi, Y.-H., H. Hu, B. Mukherjee, J. Miller, and S. K. Park. 2012. Environmental cadmium and lead exposures and hearing loss in US adults: The national health and nutrition examination survey, 1999 to 2004. *Environ. Health Perspect.* 120:1544–50. doi:10.1289/ehp.1104863.

Chou, C. F., G. L. A. Beckles, X. Zhang, and J. B. Saaddine. 2015. Association of socioeconomic position with sensory impairment among US working-aged adults. *Am. J. Public Health* 105:1262–68. doi:10.2105/AJPH.2013.301857.

Chuang, H.-Y., C.-H. Kuo, Y.-W. Chiu, C.-K. Ho, C.-J. Chen, and T.-N. Wu. 2007. A case-control study on the relationship of hearing function and blood concentrations of lead, manganese, arsenic, and selenium. *Sci. Total Environ.* 387:79–85. doi:10.1016/j.scitotenv.2007.07.032.

Chuang, H.-Y., K.-Y. Chao, and S.-Y. Tsai. 2005. Reversible neurobehavioral performance with reductions in blood lead levels – A prospective study on lead workers. *Neurotoxicol. Teratol.* 27:497–504. doi:10.1016/j.ntt.2005.01.001.

Counter, S. A., and L. H. Buchanan. 2002. Neuro-ototoxicity in Andean adults with chronic lead and noise exposure. *J. Occup. Environ. Med.* 44:30–38. doi:10.1097/00043764-200201000-00006.

Discalzi, G., D. Fabbro, F. Meliga, A. Mocellini, and F. Capellaro. 1993. Effects of occupational exposure to mercury and lead on brainstem auditory evoked potentials. *Int. J. Psychophysiol.* 14:21–25. doi:10.1016/0167-8760(93)90080-9.

Discalzi, G. L., F. Capellaro, L. Bottalo, D. Fabbro, and A. Mocellini. 1992. Auditory brainstem evoked potentials (BAEPs) in lead-exposed workers. *NeuroToxicology* 13:207–10.

Engdahl, B., K. Tambs, and H. J. Hoffman. 2013. Otoacoustic emissions, pure-tone audiometry, and self-reported hearing. *Int. J. Audiol.* 52:74–82. doi:10.3109/14992027.2012.733423.

European Agency for Health and Safety at Work. 2009. *Combined exposure to noise and ototoxic substances*. https://osha.europa.eu/en/publications/literature_reviews/combined-exposure-to-noise-and-ototoxic-substances.

Farahat, T. M., G. M. Abdel-Rasoul, A. R. El-Assy, S. H. Kandil, and M. K. Kabil. 1997. Hearing thresholds of workers in a printing facility. *Environ. Res.* 73:189–92. doi:10.1006/enrs.1997.3700.

Fazakas, Z., Z. Lengyel, and L. Nagymajtényi. 2005. Combined effects of subchronic exposure to lead, mercury and alcohol on the spontaneous and evoked cortical activity in rats. *Arh. Hig. Rada Toksikol.* 56:249–56.

Fechter, L. D., and B. Pouyatos. 2011. Correpondence: Ototoxicity. *Environ. Health Perspect.* 113:A443–A444.

Fechter, L. D., J. W. Fisher, G. D. Chapman, V. P. Mokashi, P. A. Ortiz, J. E. Reboulet, J. E. Stubbs, A. M. Lear, S. M. McInturf, S. L. Prues, et al. 2012. Subchronic JP-8 jet fuel exposure enhances vulnerability to noise-induced hearing loss in rats. *J. Toxicol. Environ. Health., Part A* 75:299–317. doi:10.1080/15287394.2012.652060.

Forst, L. S., S. Freels, and V. Persky. 1997. Occupational lead exposure and hearing loss. *J. Occup. Environ. Med.* 39:658–60. doi:10.1097/00043764-199707000-00011.

Fujimura, Y., S. Araki, K. Murata, and T. Sakai. 1998. Assessment of peripheral, central and autonomic nervous system functions in two lead smelters with high blood lead concentrations: A follow-up study. *J. Occup. Health* 40:9–15. doi:10.1539/joh.40.9.

George, L., F. Granath, A. L. V. Johansson, and S. Cnattingius. 2006. Self-reported nicotine exposure and plasma levels of cotinine in early and late pregnancy. *Acta Obstet. Gynecol. Scand.* 85:1331–37. doi:10.1080/00016340600935433.

Goswami, K., R. Gachhui, and A. Bandopadhyay. 2005. Hepatorenal dysfunctions in lead pollution. *J. Environ. Sci. Eng.* 47:75–80.

Goyer, R. A. 1997. Toxic and essential metal interactions. *Annu. Rev. Nutr.* 17:37–50. doi:10.1146/annurev.nutr.17.1.37.

Gozdzik-Zolnierkiewicz, T., and B. Moszynski. 1969. VIII nerve in experimental lead poisoning. *Acta Otolaryngol.* 68:85–89. doi:10.3109/00016486909121546.

Guthrie, O. W., B. A. Wong, S. M. McInturf, J. E. Reboulet, P. A. Ortiz, and D. R. Mattie. 2015. Inhalation of hydrocarbon jet fuel suppress central auditory nervous system function. *J. Toxicol. Environ. Health., Part A* 78:1154–69. doi:10.1080/15287394.2015.1070389.

Guthrie, O. W., H. Xu, B. A. Wong, S. M. McInturf, J. E. Reboulet, P. A. Ortiz, and D. R. Mattie. 2014. Exposure to low levels of jet-propulsion fuel impairs brainstem encoding of stimulus intensity. *J. Toxicol. Environ. Health., Part A* 77:261–80. doi:10.1080/15287394.2013.862892.

Haider, M., M. Kundi, E. Groll-Knapp, and M. Koller. 1990. Interactions between noise and air pollution. *Environ. Int.* 16:593–601. doi:10.1016/0160-4120(90)90030-A.

Hirata, M., and H. Kosaka. 1993. Effects of lead exposure on neurophysiological parameters. *Environ. Res.* 63:60–69. doi:10.1006/enrs.1993.1127.

Hoffman, H. J., R. A. Dobie, K. G. Losonczy, C. L. Themann, and G. A. Flamme. 2017. Declining prevalence of hearing loss in US adults aged 20 to 69 years. *JAMA Otolaryngol. Head Neck Surg.* 143:274–85. doi:10.1001/jamaoto.2016.3527.

Holdstein, Y., H. Pratt, M. Goldsher, G. Rosen, R. Shenhav, S. Linn, A. Mor, and A. Barkai. 1986. Auditory brainstem evoked potentials in asymptomatic lead-exposed subjects. *J. Laryngol. Otol.* 100:1031–36. doi:10.1017/S0022215100100519.

Hotta, S., T. Sugisawa, T. Matsui, T. Itoh, and K. Yamamura. 1996. Combined effects of acute lead acetate exposure and tone exposure of the guinea pig cochlea. *Eur. Arch Otorhinolaryngol.* 253:488–93.

Hughes, H., and K. L. Hunting. 2013. Evaluation of the effects of exposure to organic solvents and hazardous noise among US Air Force Reserve personnel. *Noise Health* 15:379–87. doi:10.4103/1463-1741.121224.

Huh, D.-A., Y.-H. Choi, and K. W. Moon. 2016. The effects of earphone use and environmental lead exposure on hearing loss in the Korean population: Data analysis of the korea national health and nutrition examination survey (KNHANES), 2010–2013. *PLoS One* 11:e0168718. doi:10.1371/journal.pone.0168718.

Hwang, Y. H., H. Y. Chiang, M. C. Yen-Jean, and J. D. Wang. 2009. The association between low levels of lead in blood and occupational noise-induced hearing loss in steel workers. *Sci. Total Environ.* 408:43–49. doi:10.1016/j.scitotenv.2009.09.016.

Jamesdaniel, S., R. Rosati, J. Westrick, and D. M. Ruden. 2018. Chronic lead exposure induces cochlear oxidative stress and potentiates noise-induced hearing loss. *Toxicol. Lett.* 292:175–80. doi:10.1016/j.toxlet.2018.05.004.

Jewett, D. L., and J. S. Williston. 1971. Auditory-evoked far fields averaged from the scalp of humans. *Brain.* 94:681–96. doi:10.1093/brain/94.4.681.

Johnson, A.-C., and T. C. Morata. 2010a. Chemical exposure as a risk factor for hearing loss: Implications for occupational health. *Toxicol. Lett.* 196:S3–S4. doi:10.1016/j.toxlet.2010.03.032.

Johnson, A.-C., and T. C. Morata. 2010b. Occupational exposure to chemicals and hearing impairment. *Arbete och Hälsa* 44:1–177.

Karri, V., M. Schuhmacher, and V. Kumar. 2016, December. Heavy metals (Pb, Cd, As and MeHg) as risk factors for cognitive dysfunction: A general review of metal mixture mechanism in brain. *Environ. Toxicol. Pharmacol.* 48:203–13. doi: 10.1016/j.etap.2016.09.016.

Kerr, M. J., R. L. Neitzel, O. Hong, and R. T. Sataloff. 2017. Historical review of efforts to reduce noise-induced hearing loss in the United States. *Am. J. Ind. Med.* 60:569–77. doi:10.1002/ajim.v60.6.

Khan, D. A., S. Qayyum, S. Saleem, and F. A. Khan. 2008. Lead-induced oxidative stress adversely affects health of the occupational workers. *Toxicol. Ind. Health* 24:611–18. doi:10.1177/0748233708098127.

Lalwani, A. K. 2011. Secondhand smoke and sensorineural hearing loss in adolescents. *Arch. Otolaryngol. Head Neck Surg.* 137:655–62. doi:10.1001/archoto.2011.109.

Lewis, R. C., R. R. M. Gershon, and R. L. Neitzel. 2013. Estimation of permanent noise-induced hearing loss in an urban setting. *Environ. Sci. Technol.* 47:6393–99. doi:10.1021/es305161z.

Li, X., N. Ohgami, Y. Omata, I. Yajima, M. Iida, R. Oshino, S. Ohnuma, N. Ahsan, A. A. Akhand, and M. Kato. 2017. Oral exposure to arsenic causes hearing loss in young people aged 12–29 years and in young mice. *Sci. Rep.* 7:6844. doi:10.1038/s41598-017-06096-0.

Liang, G.-H., L. Järlebark, M. Ulfendahl, J.-T. Bian, E. J. Moore, L. Ja, M. Ulfendahl, J. T. Bian, and E. J. Moore. 2004. Lead (Pb2+) modulation of potassium currents of guinea pig outer hair cells. *Neurotoxicol. Teratol.* 26:253–60. doi:10.1016/j.ntt.2003.12.002.

Lille, F., P. Hazemann, R. Garnier, and S. Dally. 1988. Effects of lead and mercury intoxicantions on evoked potentials. *Clin. Toxicol.* 26:103–16.

Liu, S., K. Zhang, S. Wu, X. Ji, N. Li, R. Liu, and X. Gao. 2011. Lead-induced hearing loss in rats and the protective effect of copper. *Biol. Trace Elem. Res.* 144:1112–19. doi:10.1007/s12011-011-9142-6.

Mac Crawford, J. M., J. A. Hoppin, M. C. R. Alavanja, A. Blair, D. P. Sandler, and F. Kamel. 2008. Hearing loss among licensed pesticide applicators in the agricultural health study. *J. Occup. Environ. Med.* 50:817–26. doi:10.1097/JOM.0b013e31816a8caf.

Mäkitie, A. A., U. Pirvola, I. Pyykkö, H. Sakakibara, V. Riihimäki, and J. Ylikoski. 2003. The ototoxic interaction of styrene and noise. *Hear. Res.* 179:9–20. doi:10.1016/S0378-5955(03)00066-2.

Mason, L. H., J. P. Harp, and D. Y. Han. 2014. Pb neurotoxicity: Neuropsychological effects of lead toxicity. *Biomed. Res. Int.* 2014:840547. doi:10.1155/2014/840547.

Masterson, E. A., P. T. Bushnell, C. L. Themann, and T. C. Morata. 2016. Hearing impairment among noise-exposed workers – United States, 2003–2012. *MMWR Morb. Mortal. Wkly. Rep.* 65:389–94. doi:10.15585/mmwr.mm6515a2.

Morata, T. C. 2003. Chemical exposure as a risk factor for hearing loss. *J. Occup. Environ. Med./Am. Coll. Occup. Environ. Med.* 45:676–82. doi:10.1097/01.jom.0000071507.96740.70.

Morata, T. C., M. Sliwinska-Kowalska, A.-C. Johnson, J. Starck, K. Pawlas, E. Zamyslowska-Szmytke, P. Nylen, E. Toppila, E. Krieg, N. Pawlas, et al. 2011. A multicenter study on the audiometric findings of styrene-exposed workers. *Int. J. Audiol.* 50:652–60. doi:10.3109/14992027.2011.588965.

Murata, K., S. Araki, K. Yokoyama, E. Uchida, and Y. Fujimura. 1993. Assessment of central, peripheral, and autonomic nervous system functions in lead workers: Neuroelectrophysiological studies. *Environ. Res.* 61:323–36. doi:10.1006/enrs.1993.1077.

Murata, K., S. Araki, K. Yokoyama, K. Nomiyama, H. Nomiyama, Y. X. Tao, and S. J. Liu. 1995. Autonomic and central nervous system effects of lead in female glass workers in China. *Am. J. Ind. Med.* 28:233–44. doi:10.1002/ajim.4700280208.

Nagymajtenyi, L., H. Schulz, and I. Desi. 1996. Electrocorticogram changes caused by subchronic lead treatment of rats. *Neurotoxicology* 17:713–17.

Neitzel, R. L., R. R. M. Gershon, T. P. McAlexander, L. A. Magda, and J. M. Pearson. 2012. Exposures to transit and other sources of noise among New York

City residents. *Environ. Sci. Technol.* 46:500–08. doi:10.1021/es2025406.

Nelson, D. I., R. Y. Nelson, M. Concha-Barrientos, and M. Fingerhut. 2005. The global burden of occupational noise-induced hearing loss. *Am. J. Ind. Med.* 48:446–58. doi:10.1002/(ISSN)1097-0274.

NIOSH (US National Institute for Occupational Safety and Health). 2018. Adult Blood Lead Epidemiology and Surveillance (ABLES). https://www.cdc.gov/niosh/topics/ables/description.html.

Osman, K., K. Pawlas, A. Schütz, M. Gazdzik, J. A. Sokal, and M. Vahter. 1999. Lead exposure and hearing effects in children in Katowice, Poland. *Environ. Res.* 80:1–8. doi:10.1006/enrs.1998.3886.

Otto, D. A., and D. A. Fox. 1993. Auditory and visual dysfunction following lead exposure. *Neurotoxicology* 14:191–207.

Park, S. K., S. Elmarsafawy, B. Mukherjee, A. Spiro 3rd, P. S. Vokonas, H. Nie, M. G. Weisskopf, J. Schwartz, H. Hu, A. Spiro, et al. 2010. Cumulative lead exposure and age-related hearing loss: The VA Normative Aging Study. *Hear. Res.* 269:48–55. doi:10.1016/j.heares.2010.07.004.

Pepler, A., K. J. Munro, K. Lewis, and K. Kluk. 2014. Prevalence of cochlear dead regions in new referrals and existing adult hearing aid users. *Ear. Hear.* 35:e99–e109. doi:10.1097/AUD.0000000000000011.

Picton, T. W., S. A. Hillyard, H. I. Krausz, and R. Galambos. 1974. Human auditory evoked potentials. I: Evaluation of components. *Electroencephalogr. Clin.Neurophysiol.* 36:179–190. doi:10.1016/0013-4694(74)90155-2.

Prasher, D. 2009. Heavy metals and noise exposure: Health effects. *Noise Health* 11:141–44. doi:10.4103/1463-1741.53358.

Rabinowitz, P., L. F. Cantley, D. Galusha, S. Trufan, A. Swersey, C. Dixon-Ernst, V. Ramirez, and R. Neitzel. 2018. Assessing hearing conservation program effectiveness. *J. Occup. Environ. Med.* 60:29–35. doi:10.1097/JOM.0000000000001125.

Raphael, Y., and R. A. Altschuler. 2003. Structure and innervation of the cochlea. *Brain Res. Bull.* 60:397–422. doi:10.1016/S0361-9230(03)00047-9.

Repko, J. D., and C. R. Corum. 1979. Critical review and evaluation of the neurological and behavioral sequelae of inorganic lead absorption. *CRC Crit. Rev. Toxicol.* 6:135–55. doi:10.3109/10408447909113048.

Rider, C. V., K. Boekelheide, N. Catlin, C. J. Gordon, T. Morata, M. J. K. Selgrade, K. Sexton, J. E. Simmons, and S. J. Rider. 2014. Cumulative risk: Toxicity and interactions of physical and chemical stressors. *Toxicol. Sci.* 137:3–11. doi:10.1093/toxsci/kft228.

Roberts, J. R., J. R. Reigart, M. Ebeling, and T. C. Hulsey. 2001. Time required for blood lead levels to decline in nonchelated children. *J. Toxicol. - Clin. Toxicol. (Phila).* 39:153–60.

Roth, J. A., and R. Salvi. 2016. Ototoxicity of divalent metals. *Neurotox. Res.* 30:268–82. doi:10.1007/s12640-016-9627-3.

Rybak, L. P. 1992. Hearing: The effects of chemicals. *Otolaryngol. Head Neck Surg.* 106:677–86. doi:10.1177/019459989210600611.

Saunders, J. E., B. G. Jastrzembski, J. C. Buckey, D. Enriquez, T. A. Mackenzie, and M. R. Karagas. 2013. Hearing loss and heavy metal toxicity in a nicaraguan mining community: Audiological results and case reports. *Audiol. Neurootol.* 18:101–13. doi:10.1159/000345470.

Sayler, S. K., P. M. Rabinowitz, D. Galusha, K. Sun, and R. Neitzel. 2018. Hearing protector attenuation and noise exposure among metal manufacturing workers. *Ear. Hear.* doi:10.1097/AUD.0000000000000650.

Schacht, J., A. E. Talaska, and L. P. Rybak. 2012. Cisplatin and aminoglycoside antibiotics: Hearing loss and its prevention. *Anat. Rec. (Hoboken)* 295:1837–50. doi:10.1002/ar.22578.

Seidman, M. D., and R. T. Standring. 2010. Noise and quality of life. *Int. J. Environ. Res. Public Health* 7:3730–38. doi:10.3390/ijerph7103730.

Simmons, J. E. 1995. Chemical mixtures: Challenge for toxicology and risk assessment. *Toxicol.* 105:111–19. doi:10.1016/0300-483X(95)03205-T.

Solenkova, N. V., J. D. Newman, J. S. Berger, G. Thurston, J. S. Hochman, and G. A. Lamas. 2014. Metal pollutants and cardiovascular disease: Mechanisms and consequences of exposure. *Am. Heart J.* 168:812–22. doi:10.1016/j.ahj.2014.07.007.

Song, F., Y. Loke, and L. Hooper. 2014. Why are medical and health-related studies not being published? A systematic review of reasons given by investigators. *PloS one* 9:e110418. doi: 10.1371/journal.pone.0110418

Spivey, G. H., C. P. Brown, R. W. Baloh, D. S. Campion, J. L. Valentine, F. J. Massey, B. L. Browdy, B. D. Culver, F. J. Massey Jr, B. L. Browdy, et al. 1979. Subclinical effects of chronic increased lead absorption – A prospective study. I. Study design and analysis of symptoms. *J. Occup. Med.* 21:423–29.

Spivey, G. H., R. W. Baloh, C. P. Brown, B. L. Browdy, D. S. Campion, J. L. Valentine, D. E. Morgan, and B. D. Culver. 1980. Subclinical effects of chronic increased lead absorption – A prospective study. III. Neurologic findings at follow-up examination. *J. Occup. Med.* 22:607–12.

Stevens, G., S. Flaxman, E. Brunskill, M. Mascarenhas, C. D. Mathers, and M. Finucane. 2013. Global and regional hearing impairment prevalence: An analysis of 42 studies in 29 countries. *Eur. J. Public Health* 23:146–52. doi:10.1093/eurpub/ckt147.

Sun, D. Q., X. Zhou, F. R. Lin, H. W. Francis, J. P. Carey, and W. W. Chien. 2014. Racial difference in cochlear pigmentation is associated with hearing loss risk. *Otology. And Neurotology.* 35:1509–14. doi:10.1097/MAO.0000000000000564.

Takahashi, Y., Y. Okamoto, and K. Saito. 1984. Electrocorticogram (ECoG) and auditory evoked potential (AEP) in rats intoxicated with lead acetate. *Ind. Health* 22:189–98. doi:10.2486/indhealth.22.189.

Tharpe, A. M., D. P. Sladen, J. Dodd-Murphy, and S. J. Boney. 2009. Minimal hearing loss in children:

Minimal but not inconsequential. *Seminars in Hearing* 30 (2): 80–93.

Tuncel, U., W. J. Clerici, and R. O. Jones. 2002. Differential ototoxicities induced by lead acetate and tetraethyl lead. *Hear. Res.* 166:113–23. doi:10.1016/S0378-5955(02)00303-9.

Vos, T., . R. M., B. Barber, A. Bell, S. Bertozzi-Villa, I. Biryukov, F. Bolliger, A. Charlson, L. D. Davis, and C. J. L. Murray. 2015. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: A systematic analysis for the Global Burden of Disease Study 2013. *The Lancet* 386:743–800. doi:10.1016/S0140-6736(15)60692-4.

Wake, M., E. K. Hughes, Z. Poulakis, C. Collins, and F. W. Rickards. 2004. Outcomes of children with mild-profound congenital hearing loss at 7 to 8 years: A population study. *Ear. Hear.* 25:1–8. doi:10.1097/01.AUD.0000111262.12219.2F.

Wilker, E., S. Korrick, L. H. Nie, D. Sparrow, P. Vokonas, B. Coull, R. O. Wright, J. Schwartz, and H. Hu. 2011. Longitudinal changes in bone lead levels: The VA normative aging study. *J. Occup. Environ. Med.* 53:850–55. doi:10.1097/JOM.0b013e31822589a9.

Wilpizeski, C. 1974. Effects of lead on the vestibular system: Preliminary findings. *Laryngoscope* 5:821–32. doi:10.1288/00005537-197405000-00013.

World Health Organization. 1991. *Report of the informal working group on prevention of deafness and hearing impairment programme planning*. Geneva, Switzerland: World Health Organization Press.

World Health Organization. 2008. *The global burden of disease: 2004 update*. Geneva, Switzerland: World Health Organization Press.

World Health Organization. 2018. Prevention of blindness and deafness: Estimates. http://www.who.int/pbd/deafness/estimates/en/.

Wu, T., C. Shen, J. Lai, C. Goo, K. Ko, H. Y. Chi, P. Y. Chang, and S. H. Liou. 2000. Effects of lead and noise exposures on hearing ability. *Arch. Environ. Health* 55:109–14. doi:10.1080/00039890009603396.

Yamamura, K., K. Terayama, N. Yamamoto, A. Kohyama, and R. Kishi. 1989. Effects of acute lead acetate exposure on adult guinea pigs: Electrophysiological study of the inner ear. *Fundam. Appl. Toxicol.* 13:509–15. doi:10.1016/0272-0590(89)90287-X.

Yamamura, K., N. Maehara, K. Terayama, N. Ueno, A. Kohyama, Y. Sawada, and R. Kishi. 1987. Effects of lead acetate on guinea pig-Cochear microphonics, action potential, and motor nerve conduction velocity. *Bull Environ. Contam. Toxicol.* 38:571–79. doi:10.1007/BF01608588.

Yamamura, K., R. Kishi, N. Maehara, T. Sadamoto, and E. Uchino. 1984. An experimental study of the effects of lead acetate on hearing. Cochlear microphonics and action potential of the guinea pig. *Toxicol. Lett.* 21:41–47. doi:10.1016/0378-4274(84)90221-2.

Yokoyama, K., S. Araki, K. Yamashita, K. Murata, K. Nomiyama, H. Nomiyama, Y. X. Tao, and S. J. Liu. 2002. Subclinical cerebellar anterior lobe, vestibulocerebellar and spinocerebellar afferent effects in young female lead workers in China: Computerized posturography with sway frequency analysis and brainstem auditory evoked potentials. *Ind. Health* 40:245–53. doi:10.2486/indhealth.40.245.

Zheng, Q. Y., K. R. Johnson, and L. C. Erway. 1999. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. *Hear. Res.* 130:94–107. doi:10.1016/S0378-5955(99)00003-9.