Latent consequences of early-life lead (Pb) exposure and the future: Addressing the Pb Crisis

Bryan Maloney,[1,3] Baindu L. Bayon,[1,2] Nasser H. Zawia,[4] and Debomoy K. Lahiri[1, 2,3*]

[1]Departments of Psychiatry and [2]Medical & Molecular Genetics, Stark Neurosciences Research Institute, [3]Indiana Alzheimer Disesae Center, Indiana University School of Medicine, Indianapolis, IN, USA; [4]Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, USA

*Corresponding Author

Dr. D.K. Lahiri

Department of Psychiatry, Neuroscience Research Building

Indiana University School of Medicine

320 W. 15th St., Indianapolis IN 46202, USA

Phone: (317) 274-2706

dlahiri@iupui.edu

Author email addresses:

Baindu L. Bayon: bbayon@gmail.com

Bryan Maloney: brmalone@iupui.edu

Nasser H. Zawia: nzawia@uri.edu

Debomoy K. Lahiri: dlahiri@iupui.edu

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Abstract

Background. The lead (Pb) exposure crisis in Flint, Michigan has passed from well-publicized to a footnote, while its biological and social impact will linger for a lifetime. Interest in the "water crisis" has dropped to pre-event levels, which is neither appropriate nor safe. Flint's exposure was severe, but it was not unique. Problematic Pb levels have also been found in schools and daycares in 42 states in the USA. The enormity of Pb exposure via municipal water systems requires multiple responses. Herein, we focus on addressing a possible answer to *long-term* sequelae of Pb exposure. We propose "4R's" (remediation, renovation, reallocation, and research) against the Pb crisis that goes beyond a short-term fix. Remediation for affected individuals must continue to provide clean water and deal with both short and long-term effects of Pb exposure. Renovation of current water delivery systems, at both system-wide and individual site levels, is necessary. Reallocation of resources is needed to ensure these two responses occur and to get communities ready for potential sequelae of Pb exposure. Finally, properly focused research can track exposed individuals and illuminate latent (presumably epigenetic) results of Pb exposure and inform further resource reallocation.

Conclusion. Motivation to act by not only the general public but also by scientific and medical leaders must be maintained beyond initial news cycle spikes and an annual follow-up story. Environmental impact of Pb contamination of drinking water goes beyond one exposure incident in an impoverished and forgotten Michigan city. Population effects must be addressed long-term and nationwide.

Abbreviations. CDC: Centers for Disease Control (USA); DNA: deoxyribonucleic acid; EPA: Environmental Protection Agency (USA); GCF: Great Chinese Famine; HDACi; Histone deacetylase inhibitors; miRNA: microRNA; Pb: Lead; ppb: Parts per billion; RNA: ribonucleic acid; tLEARn: Transgenerational Latent Early-Life Regulation

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Introduction

In 2014, over 100,000 residents of the city of Flint, Michigan, were potentially exposed to high levels of lead (Pb) via drinking water from a contaminated water source, their own water system's pipes, leached out by improperly-treated source water. This contamination led to declaration of a federal state of emergency by President Barack Obama in 2016 and widespread attention to this state and national public health catastrophe. Pb is a known neurotoxin that adversely affects neurodevelopment and cognition (Santa Maria et al., 2018). In addition, prolonged Pb exposure can cause digestive problems, renal issues, as well as anemia (Nemsadze et al., 2009; Yilmaz et al., 2012). Pb can substitute for calcium ions (Ca^{++}), which makes it a disruptor of Ca^{++} homeostasis. This can leads to an accumulation of Ca^{++} in cells, affecting the activity of secondary messengers (Bressler et al., 1999). This disruption can cause apoptosis and changes in excitotoxicity and neurotransmitter release and storage. The incidence of Pb poisoning from cumulative Pb exposure shows a dose-response relationship (Wu et al., 2016). In short, the toxicity of

acute and chronic Pb exposure are dramatic and devastating. However, these strictly "toxic" responses are not the sum total of the dangerous sequelae of Pb exposure and may not even be the most serious long-term consequences.

Pb Exposure Causes Epigenetic Changes

These long-term consequences are likely to be maintained within the body as changes in epigenetic markers. In the present day, "epigenetics" has at times been broadly defined, perhaps beyond meaning. Herein, we restrict it to "phenomena and mechanisms that cause chromosome-bound, potentially heritable changes in gene expression that are not changes in the DNA sequence" (Deans and Maggert, 2015). Biochemically, this would include DNA oxidation (Zawia et al., 2009), methylation (Sen et al., 2015b), and hydroxymethylation (Sen et al., 2015a) patterns, and histone modifications (such as acetylation) (Xu, L.H. et al., 2015). It is likely that such epigenetic "lesions" are responsible for a large number of the latent sequelae to Pb exposure, resulting in genome-wide changes of DNA methylation and expression profiles (Dosunmu et al., 2009). The contribution of epigenetic dysfunction to late-life neurodegeneration is well-attested (Maloney and Lahiri, 2016). In the realm of DNA methylation, this damage may occur due to disruption of the methionine-homocysteine cycle, wherein Pb exposure elevates levels of homocysteine (Brucker et al., 2015; Yakub and Iqbal, 2010) and by reprogramming expression levels of enzymes that regulate epigenetic DNA and histone modification (Eid et al., 2016). Homocysteine contributes both to aberrant DNA methylation (Lin et al., 2014; Perng et al., 2014; Yang et al., 2014) and to altered histone acetylation (Xu, L. et al., 2015). Furthermore, early-life Pb exposure may be linked to Alzheimer's disease (AD) (Wu et al., 2008). In particular, early-life exposure to low (sub-toxic) levels of Pb can induce late-life aberrations in expression of AD-associated proteins and peptides, even though late-life Pb levels were the same as for un-exposed individuals (Basha et al., 2005b; Wu et al., 2008). These latent abnormalities are most likely preserved through epigenetic means (Bolin et al., 2006; Zawia et al., 2009), and later-life triggers interact with these altered epigenetics to produce clinical abnormalities (Maloney and Lahiri, 2016). In addition to altering DNA modification, Pb may increase histone acetylation in some tissues (Li-Hui Xu, 2015). Also of great concern is that environmentally-induced epigenetic lesions may be passed across generations, leading to familial predisposition to neuropsychiatric disorders, as we have previously discussed in more detail (Lahiri et al., 2016). Finally, in addition to epigenetic factors, Pb exposure alters levels of microRNA (miRNA) species that target proteins associated with disease (Masoud et al., 2016), although this is not as well elucidated and may be due to epigenetic modification of the miRNA genes' own regulatory sequences.

Chelation Does Not Prevent Long-term Effects.

An unfortunately popular response was to call for chelation therapy for all exposed children (2016a; 2016b; Conat, 2016; Environews DC News Bureau, 2016; Trowbridge, 2014). Some press outlets did note that this approach previously proved ineffective (Lapook, 2016), and public health experts also counseled against this approach (Reilly, 2016; Wade, 2016; Young, 2016), Nevertheless, even some of these public statements were watered down by publishers, who thought it appropriate to "balance" legitimate experts with counter-statements from "chiropractic neurologists" (Young, 2016). Chelation might reduce levels of circulating Pb, but the reduction offers little to no benefit to any cognitive, neuromotor, or behavioral endpoints (Dietrich et al., 2004). In any case, even the legitimate public health response (e.g., high-ascorbate/calcium/iron diet) was geared toward preventing chronic accumulation (Reilly, 2016; USDA Office of Communications, 2016; Wade, 2016), *not the sort of latent sequelae we describe*. One might

conclude that the results of Pb exposure would be, therefore, insuperable, since removal of the Pb from the body does not reverse outcomes of exposure.

Pb Exposure Requires Timely Intervention

There is, nevertheless, a viable response to long-term sequelae of Pb exposure, because there is a biochemical mechanism for this long-term, latent Pb damage, i. e., long-term effects of disruption of epigenetic markers. Thus, epigenetic intervention may be possible. Epigenetic drugs currently under human trial, such as resveratrol, a histone deacetylase inhibitor (HDACi) (Vahid et al., 2015), also regulate the homocysteine cycle (Koz et al., 2012) and may likewise have cognitive benefits (Koz et al., 2012). Resveratrol may be able to perform double-duty against Pb-induced latent hazards, given that it can regulate both homocysteine and histone acetylation. Dietary supplementation with vitamins B6, B12, and folate may reverse several epigenetic effects of homocysteine activity (Kok et al., 2015). In particular, such supplementation accompanies reduced decline or even improvement in neurological patient samples (Chan et al., 2010; Kim et al., 2014; Lahiri and Maloney, 2010; Reynolds, 2014), although it should be cautioned that response to B vitamin supplementation may be subject to significant genetic influence (Chhillar et al., 2014).

Consequences of Environmental Catastrophes have Complex Health Consequences.

Environmental exposures certainly affect subgroups differently, and the disparity among those groups is notable. A life course model proposes that the high infant mortality rates of Blacks vs non-Hispanic whites is due to a higher number of multifactorial risk factors from conception to death (Lu and Halfon, 2003).

While Pb exposure is classically associated with learning difficulties, hyperactivity, and developmental delays, some of which may be irreversible and current public concern was and remains over these specific pediatric and later behavioral difficulties (Ungar, 2016), which were recognized decades ago (De la Burde and Choate, 1972). There are far deeper and longer-lasting effects of Pb exposure. Multiple latent sequelae exist of Pb exposure to *sub-toxic* levels (Bakulski, 2013; Basha et al., 2005a; Basha et al., 2005b; Bihaqi et al., 2014; Bolin et al., 2006; Chiu et al., 2014; Eid et al., 2016; Masoud et al., 2016; Sen et al., 2015a; Sen et al., 2015b; Wu et al., 2008). Pb exposure can instill *latent* epigenetic modifications (Zawia et al., 2009) that occur in the *absence* of toxicity symptoms. This is the basis of the Latent Early-Life Regulation (LEARn) model that has been expanded recently (Maloney and Lahiri, 2016). Briefly, LEARn posits that individual "hits" can occur early in life. These can take the form of exposure to toxic materials (such as Pb), to environmental stress (such as poverty or racial disparity), to cultural stress (such as warfare), or other negative impacts. Each hit has the potential to impose epigenetic changes, such as DNA methylation (Maloney and Lahiri, 2016) or proliferation of long, interspersed nuclear element (LINE)1 transposons within brain regions such as the hippocampus (Bedrosian et al., 2018; Lahiri et al., 2018). If sufficient hits are suffered before a critical age threshold, risk of developing a later-life disorder, such as AD, greatly increases (Fig. 1A). Multiple dementias have strong epigenetic connections, both abnormal histone modification and DNA methylation (Maloney and Lahiri, 2016). Several specific loci differences in DNA methylation associate with AD (De Jager et al., 2014). Epigenetic connections also exist to Parkinson's disease (Feng et al., 2015), frontotemporal dementia/other tauopathies (Li et al., 2014), and overall mortality (Marioni et al., 2015).

These epigenomic effects of environmental hazards are not limited to a single generation. Epigenetic changes, including those with behavioral effects, can be inherited (Anway et al., 2005; Bygren, 2013; Dias and Ressler, 2014; Stegemann and Buchner, 2015; Vassoler and Sadri-Vakili, 2014; Vassoler et al., 2013; Wei et al., 2014; Xie et al., 2018). Combining this possibility with LEARn produces t-LEARn (transgenerational LEARn) (Lahiri et al., 2016). In essence, there may be a significant risk of passing down the latent *consequences* of "sub-toxic" Pb exposure to future generations (Fig. 1B), even though the future generations are not exposed to elevated Pb (Lahiri et al., 2016). Multigenerational effects are not like simple prenatal exposure. A Detroit population was surveyed for aberrant DNA methylation. Subjects were grandmothers, mothers, and grandchildren. A woman were exposed to Pb was more likely to have aberrant DNA methylation for specific genes in her, in her daughter's, and in her grandchild's DNA. These genes included ninjurin2 (NINJ2) and N-myc downstream-regulated gene 4 (NDRG4) (Sen et al., 2015c), both of which are associated with AD (Lin et al., 2011; Zhou et al., 2001), as well as apolipoprotein A5 (APOA5), which is associated with hypertriglyceridemia, a cardiovascular risk factor (Caussy et al., 2014).

In addition to general considerations, it is important to continue to consider the unique impact the Flint water crisis will continue to have on a predominantly African-American population, given particular risk associations that exist between this group and later-life neurodegeneration (Barnes and Bennett, 2014), and investigate risk factor disparities vs. other ethnic and racial groups. Furthermore, it will be key to explore the range of long term mental health effects of chronic Pb exposure combined with the stress of poverty and other environmental factors on the people of and *from* Flint. Future outcomes of unemployment and unstable family structures could be associated with poor mental health among those children not only exposed to high Pb levels for extended periods of time, but exacerbated by limited social support and institutionalized stigma. These risk factors contribute to poor mental health outcomes in youth affected by other traumatic experiences such as war (Sharma et al., 2017). Coping strategies and mechanisms employed by those who have suffered crises such as famine, war, and forced migration may be useful in building adaptive and prosocial behaviors in Flint youth in order to recover from such a devastating early life exposure. However, behavioral therapy would only have limited effect in the face of neurobiological alterations brought about by Pb exposure.

Flint's Water Problem Was Not Unique.

The Centers for Disease Control (CDC) sets 5µg/dl blood Pb as the actionable value. By one estimate, frequency of children in Flint under six years of age with blood Pb levels above 5µg/dl fell from 16.2% in 2005 to 3.6% in 2013, at which point it was on par with the USA as a whole. Upon switching to the more corrosive water from the Flint River, which leached Pb from the water system, levels rose to 6.4% by 2015 (Drum, 2016). More in-depth investigation established pre- and post-source change levels at 2.4% vs. 4.9% in Flint in general, with high Pb level areas of Flint starting from 4% in 2013 and elevated to 10.6% in 2015 (Hanna-Attisha et al., 2016).

The majority of the world has simply moved on. This is neither ethical nor safe. Flint's exposure was severe, but it is not unique. Troubling Pb levels were found in drinking water sources in schools and daycares in 42 states in the USA (Ungar, 2016). The current public health goal, regardless of official standards, is no Pb content: No level of Pb exposure is non-toxic (Environmental Protection Agency (EPA) USA, 2016; Ungar, 2016). Testing the water at sinks and water fountains revealed multiple instances at more than 100 ppb, with the highest being a 5,000 ppb sample from the restroom sink at Caroline

Elementary School in Ithaca, NY (Ungar, 2016). Putting this situation into even bleaker perspective, no national mandate in the U.S. exists for Pb testing specifically at schools or daycares (EPA USA, 2016), and not all states require such testing. While city-wide "water systems" are required to test for Pb at the tap, and if more than 10% of samples fall above 15 ppb, a water system is required to take action, a system that serves 100,000 or more people need test at no more than 100 locations (EPA USA, 2016), To exacerbate this issue, multiple US cities have used testing "cheats" that conceal dangerous levels of Pb (Gajanan, 2016). These "cheats" include pre-flushing pipes before testing, removal of aerators, and running water slowly for test purposes.

Beyond water: Pb Exposure Originates from Various Sources.

Pb exposure through drinking water is far from the only significant source of exposure to environmental Pb. Although leaded gasoline and paint have now been eliminated from the market, paint that contains Pb is not routinely removed from older dwellings. Pb also adheres to soil and persists for extended periods. Such soil-bound Pb may be a significant source of exposure for children in many urban areas (Frazer, 2008). Measurement of Pb exposure in several urban areas at least a decade after the bans on leaded gasoline and paint were enacted showed effects of temperature and soil moisture on pediatric blood Pb levels. Inner-city children had higher blood Pb levels in late summer months and lower levels in winter (Laidlaw et al., 2005). Soil Pb vs. pediatric blood Pb levels were explicitly modeled for New Orleans, and an extrapolation of changing soil Pb limits from 400 mg/kg to 100mg/kg predicted an economic benefit of approximate $4,700 to $12,500 per child (year 2000 dollars) (Zahran et al., 2011). A neighborhood's past can continue to haunt for decades. For example, the Indiana city of South Bend repeatedly shows high levels of Pb in tests of children from the northwest side of the city. This area has a concentration of homes with Pb-containing paint (Associated Press, 2018; Booker, 2018). The longstanding ban on Pb-containing paint has done little to nothing to eliminate it from older housing, thus, people are still exposed. According to the CDC, other sources of Pb include toys, candy imported from Mexico and artificial turf (Centers for Disease Control and Prevention USA, 2015).

Developing a Larger Solution: Remediation, Renovation, Reallocation* and *Research

The lead (Pb) exposure crisis in Flint, Michigan, USA has passed from an initial outcry, a feeling of "crisis" to merely a footnote. A crisis is to be dramatic and isn't expected to last for years, however in the case of Pb, effects last long after feelings of urgency pass. Of course, the short memory of the general public does not mean that the scientific community has any obligation to go with such a flow.

We propose "4R's" (Fig. 2) against the Pb crisis that goes beyond any quick fix. Acute remediation must, of course, be done first to address acute symptoms. Flint residents need to have clean water. In parallel, water systems need to be renovated, including adding anti-corrosive agents to water. Pb-containing components would be removed and replaced, possibly on a home-by-home basis. Steps to reduce overall corrosiveness of water supplies could reduce risk in systems while they are being updated. All of these measures require reallocation of considerable resources. Administrators of water systems need to be made aware not only of regulatory requirements but how regulation may fall short of the needs of their community. Bare adherence to the letter of the law does not guarantee safety from dangerous levels of Pb. Willingness to accurately measure Pb concentration and exposure is necessary. Proper Pb level quantification and monitoring need to be modernized to account for water systems that far exceed the capacity and volumes envisioned when rules were originally formulated. Methods must be

standardized and standards *enforced* to limit Pb exposures (Rosen et al., 2017). All of these measures are likely to prove inadequate to the so-called epigenetic "time bomb" that was set in Flint. It will not be enough to clean up Flint's water. In addition to acute "remediation", attention must be paid to discovering and possibly reversing these latent epigenetic lesions, which may consist of aberrant DNA methylation, disruption of histone modification, and other markers. While there is research on promising epigenetic remediation (Fig. 1C), such as resveratrol (Wang et al., 2016) or dietary supplementation with S-adenosylmethionine (Chan and Shea, 2006) or folic acid (Bae et al., 2014; Kok et al., 2015), it is still far from conclusive.

Providing 'Remediation' and 'Renovation' will require money, be it by tax credits, loans, or outright grants to water system operators, municipalities, school systems, or individual homeowners. Fortunately, some movement has occurred. Millions of dollars of grants have been allocated to research and renovation for Flint (Community Foundation Greater Flint, 2018; EPA USA, 2017; Mott Foundation, 2016) and South Bend (Booker, 2017). However, this is still a piecemeal approach to what appears to be a much larger, if latent, problem.

Once potential "Pb hotspots" have been identified, public health and educational resources may need to be mobilized to deal with long-term effects of exposure. Underpinning and supporting all of these efforts would be focused research. It should not be limited to a single approach or discipline. Epigenetic monitoring of exposed *individuals* is certainly important, particularly in correlation with development of later-life neurological conditions, potential dietary modification of their epigenomes, etc. However, monitoring exposed *communities* is also necessary, not only to ensure regulatory compliance, but to track which local policy decisions end up significantly preventing Pb exposure over the long term. These research results, of course, could then be used to inform future resource reallocation decisions. This research would need to be conducted in a culturally sensitive manner, considering the vulnerability of the population and justifiable public mistrust, further exacerbated by an unresponsive government that has devastated so many. In any case, an integrated approach of all of these factors will likely be necessary to meet not only the challenges posed by Pb exposure, but by the ever-increasing number of man-made environmental challenges (e.g., pesticides) that also have been found to exert a significant long-term public health and epigenomic effect.

Flint, Michigan is a city of nearly 100,000 with a majority of African-American citizens, making up nearly 57% of its population (US Census Bureau, 2017). Complaints from Flint residents concerning skin problems and changes in the appearance of their water at most evoked boil water advisories (Lin, 2016). When Pb levels in Flint's tap water were tested and found to be more than twice the maximum contaminant level classification of the EPA (Craft-Blacksheare, 2017). Socioeconomic disparity is a fundamental element in this crisis. Even though Flint residents, on average, pay more than other citizens in the United States for their water (Lynch, 2016), they have essentially been poisoned by it, further emphasizing disparity on outcome vs. prices assessed to individual residents. Institutional problems are still entrenched in Flint governance. Flint's water situation is neither strictly biomedical nor limited to a single city. It is a large-scale public health issue that particularly infests low-income, low-status communities. The scientific community could take a lead in addressing such issues.

We hope that we have provided a useful model to transcend this sole event and that can be applied to other sources of intoxication, other toxicants, and other mechanisms of action. We wish for readers to consider the full dimensions of the problem and seriously consider the long-term

socioeconomic consequences of a presumably isolated episode of intoxication. Such awareness deserves to be known worldwide, not only to the research community (who takes part in one R) but also to regulatory agencies and decision makers who are involved with the other 3 Rs.**Declarations.** The authors declare they have no competing financial interests, have contributed equally to the article.

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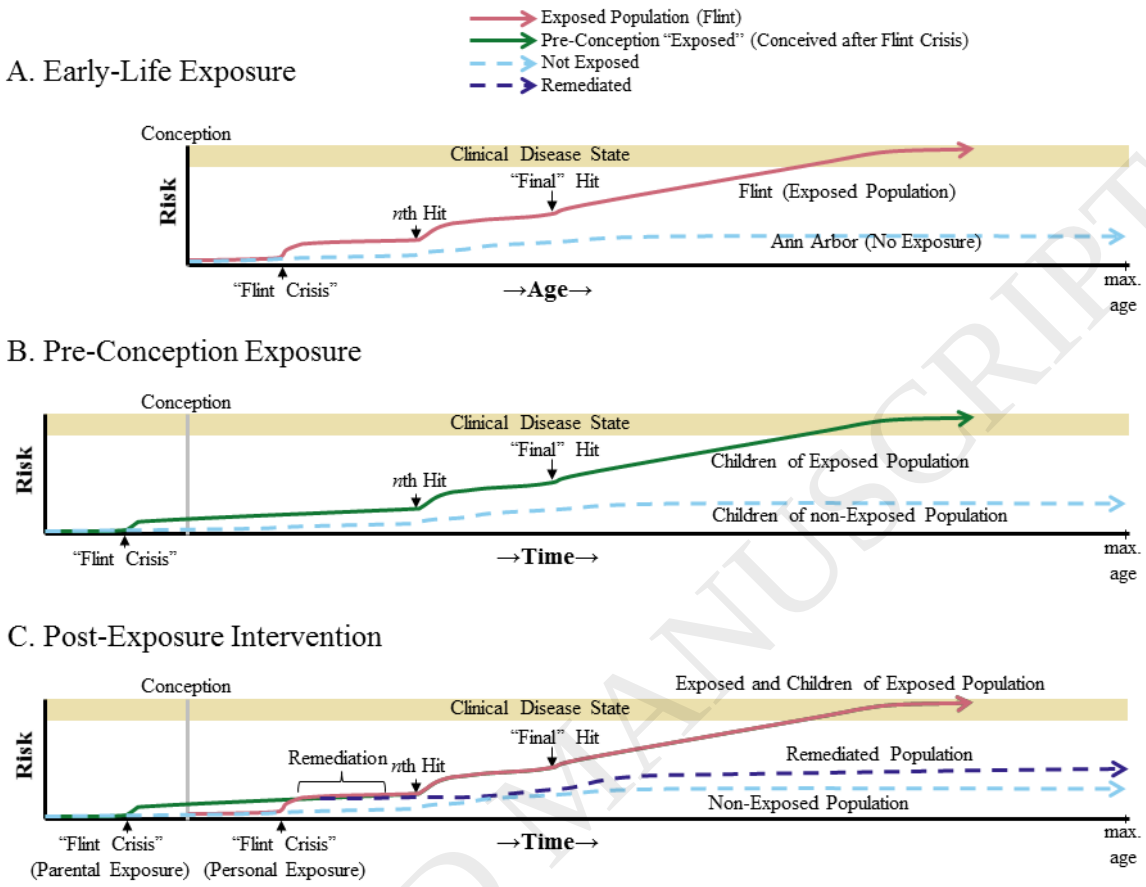
Figure Legends

Fig. 1. The LEARn Models and Flint. LEARn explains idiopathic disorders in a testable manner, on the basis of accumulation of "hits" through an organism's lifespan. Hits can be environmental, genetic, or epigenetic. A) An event such as a Pb/water exposure crisis would be the critical "initial hit" that sets up latent increased risk (red line) for late-life neurodegenerative disorders. As additional hits are received, people who had the primary hit progress to clinical neurodegeneration. People who did not have the critical early-life hit (un-exposed, dashed blue line) do not progress to disease, even though they also might bear later hits. B) The critical hit may occur before conception. Environmentally-induced epigenetic changes can be inherited, thus, even though individuals may be born after an acute "water crisis" has passed and suffer no direct exposure, the *results* of that exposure could still be passed on epigenetically and act as the critical primary hit (green line). C) In either case, appropriate remediation at an early enough interval may reverse epigenetic lesion in either primary-exposed individuals or their descendants (violet line). However, finding such lesions and appropriate remediation will require longitudinal research of affected individuals and their offspring.

Fig. 2. Proposal of four R's of response to widespread Pb contamination. The enormity of Pb exposure via municipal water systems requires multiple responses. Immediate Remediation must be done for affected individuals, in terms of providing clean water and dealing with both short and long-term effects of Pb exposure. Renovation of current water delivery systems, at both system-wide and individual site levels, is necessary. Reallocation of resources is necessary to ensure these two responses occur and to get communities ready for potential sequelae of Pb exposure. Research, properly focused can track exposed individuals and illuminate latent (presumably epigenetic) results of Pb exposure and inform further resource Reallocation.

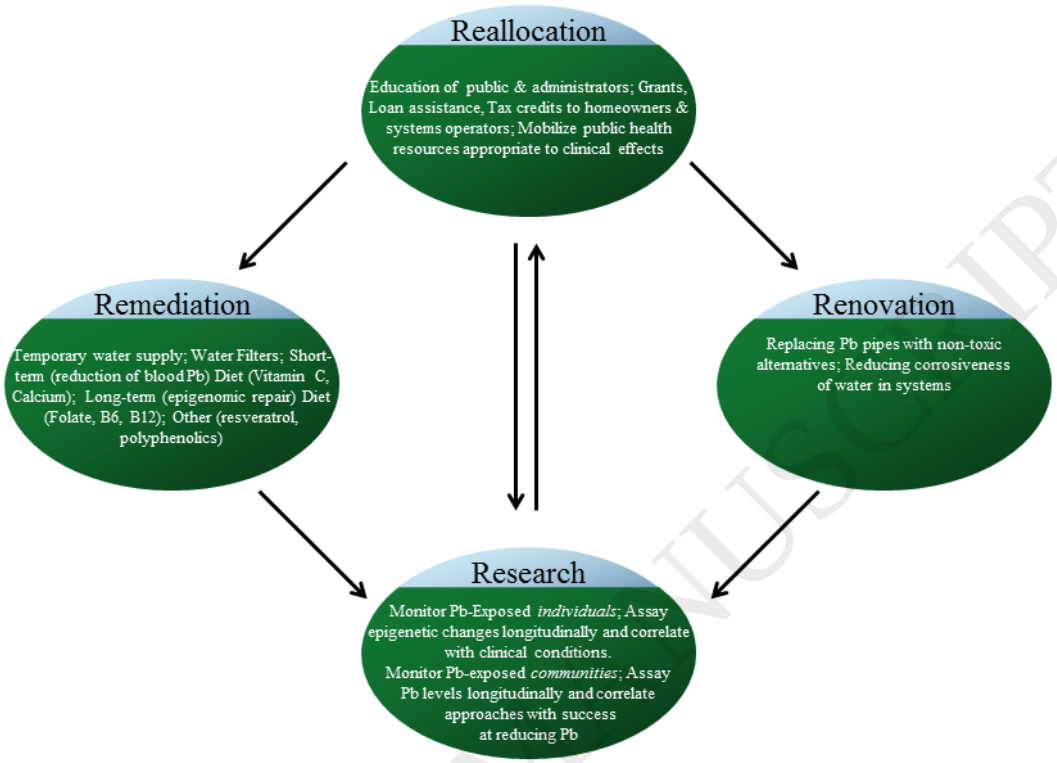
Figr-1

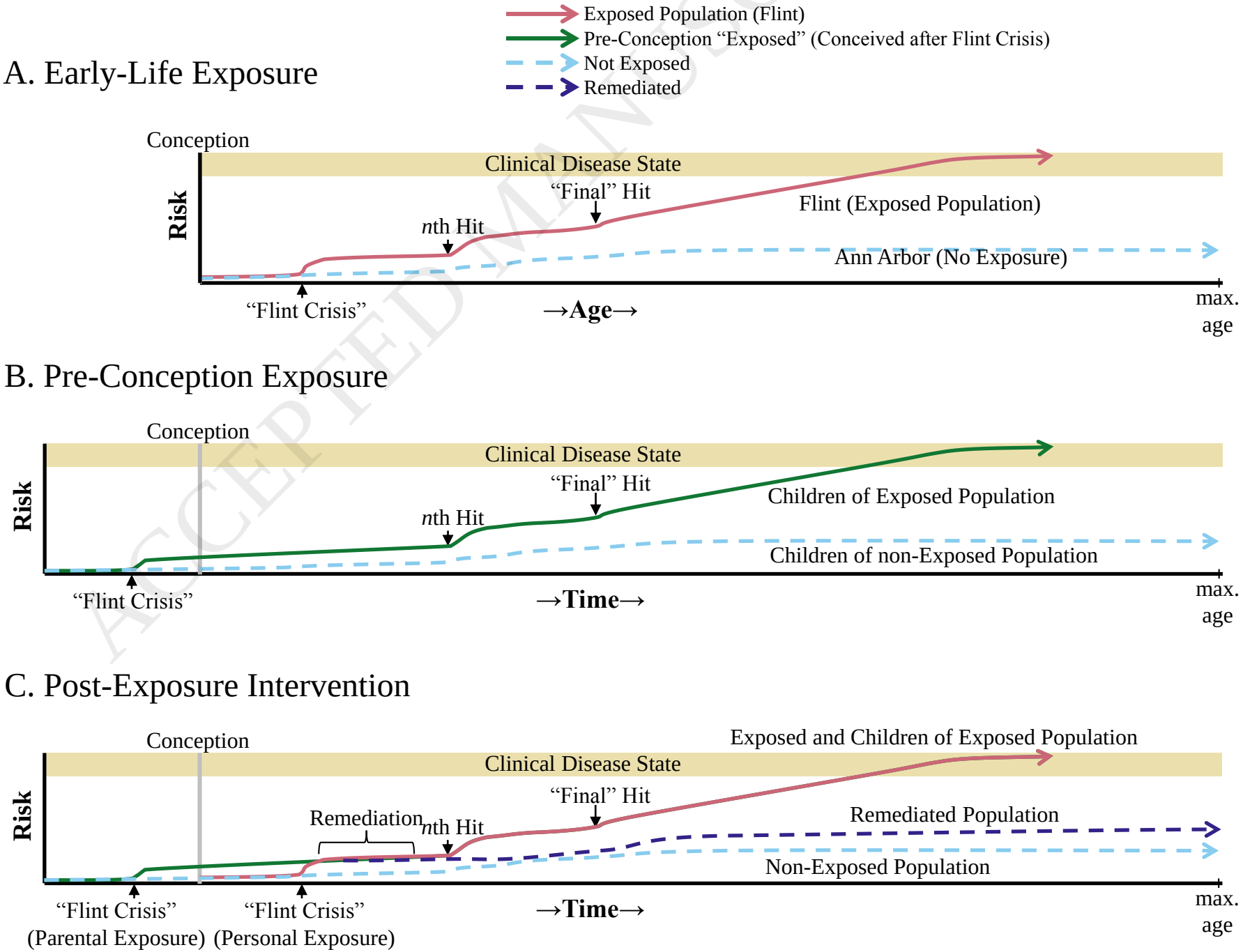
1



Figr-2

2





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