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Functional consequences of lead and mercury exposomes in the heart

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ABSTRACT

Lead and mercury are heavy metals that are highly toxic to life forms. There are no known physiological processes that require them, and they do not have a particular threshold concentration to produce biologic damage. They are non-biodegradable, and they slowly accumulate in the environment in a dynamic equilibrium between air, water, soil, food, and living organisms. Their accumulation in the environment has been increasing over time, because they were not banned from use in anthropogenic industrial production. In their +2 cationic state they are powerful oxidizing agents with the ability to interfere significantly with processes that require specific divalent cations. Acute or chronic exposure to lead and mercury can produce multisystemic damage, especially in the developing nervous systems of children and fetuses, resulting in variety of neurological consequences. They can also affect the cardiovascular system and especially the heart, either directly through their action on cardiomyocytes or indirectly through their effects on innervation, humoral responses or blood vessel alterations. For example, heart function modified by these heavy metals are heart rate, contraction, excitability, and rhythm. Some cardiac molecular targets have been identified and characterized. The direct mechanisms of damage of these heavy metals on heart function are discussed. We conclude that exposome to these heavy metals, should be considered as a major relevant risk factor for cardiac diseases.

1. Introduction

After the completion of the human genome project, it became evident that there was a need for an epidemiological definition of the non-genetic factors that lead to disease. Professor Christopher Paul Wild defined the exposome as a concept that complements the genome and includes the entire external and internal environmental human exposure to non-genetic factors from conception until a certain age, and how it affects health and promotes disease (Wild, 2005, 2012). The concept of exposome can be approximated by producing a system that estimates interacting natural or anthropogenic environmental pollutants that are epidemiologically relevant in space and time and that interact with each other and various ecosystems, resulting in environmental damage and specific harm to public health from conception to all ages (Chung et al., 2018; Lucock and Medicine, 2020; Tamayo-Uria et al., 2019). A useful epidemiological approach has been to measure reliable all of the relevant environmental variables of lead and mercury exposures using biomarkers, sensors, among others, and then to characterize the potential molecular effects of these heavy metal pollutants.

Metals are elements that can play an essential role in the human exposome, because they can directly or indirectly modulate membrane proteins and especially enzymes whose activities depend on their presence and/or modulate pathways related to, for example, redox signaling and calcium signaling (Sigel et al., 2013). They can be classified as:*a)*

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Metal ions of physiological importance, and which require a minimal concentration to sustain life but are toxic when found in excess (some examples,Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}), *b)* Non-physiological metal ions with a low environmental impact and that are generally of low toxicity (i.e. Ba^{2+}, Sr^{2+}, Co^{2+}, Ni^{2+}, and others), and finally, *c)* Non-physiological metal ions with a high environmental impact but without a physiological role that are always toxic to cells and their exposome is part of a public health issue worldwide (mostly heavy metals such as lead and mercury). The last category is particularly relevant because *i)* when found, it implies the presence of an exposome, *ii)* Lead and mercury are highly toxic, and they accumulate in many cells and tissues, *iii)* they are non-biodegradable, and hence *iv)* they generate environments in which they accumulate to form a dynamic equilibrium between water, soil, air, polluted food, and living organisms (Fig. 1). Heavy metals are key players in causing disease in humans and other living forms, and constitute important exposomes that need to be carefully studied and monitored worldwide (Callaway, 2012). Among the most commonly found toxic heavy metals, lead and mercury are elements that meet these criteria. Both, lead and mercury, accumulate persistently in the environment, because they are not biodegradable. They generate exposomes that alter ecosystems (including human), and they exist in a dynamic equilibrium of various concentrations distributed between soil, air, water, food, and various organisms (Fig. 1). The arrows in Fig. 1 represent the transfer from one state to another, as if in chemical equilibrium, with the peculiarity that the constants and values are dependent on physicochemical and biological interrelations between each medium for a particular space (and time). The circular arrows represent the ability of some forms (especially Hg) to interconvert between each chemical form. This interplay creates a network map that defines the core sections within the exposome and how they are related to each other. In dealing epidemiologically with these heavy metal exposomes requires simultaneous monitoring of all environments in space and time. Studying the effects of lead and mercury exposomes in people and other living forms, constituting various ecosystems with various units of exposure that promote particular damage or disease, can be thought of in a similar way to the research on radiation exposures. Considering that i) exposure to lead and mercury is usually done in small amounts and from time to time, and that ii) metals are stably stored mainly in bone; like it happens with radiation exposure, the exposition to these heavy metals even in small amounts can produce damage, having no threshold to induce harm. Their accumulation is progressive, reaching toxic levels after years of exposure. Hence, as it happens with radiations, there are no safe limits of exposure for these metals. The scientific community has approached this problem by the use of independent, non-synchronized studies on the distribution of these heavy metals in living forms and various environments, for example, in studies on the toxic effects of lead (Cooper and Wong, 1985; Jaradat and Momani, 1999; Morgan, 1994; Nabulo et al., 2006; Sharpe and Livesey, 2006; Zook et al., 1972). These heavy metal exposomes require elimination which can take decades, and active human policies are needed to erradicate them. They are an important cause of toxicity worldwide producing widespread toxicity and poisoning (Gidlow, 2015; Sakamoto et al., 2018).

This review will focus on the functional impact of lead and mercury exposures on the heart. We will discuss general issues on their chemistry, and then review their effects.

2. Lead and mercury: their chemistry, poisoning general concepts, experimental approaches to study their direct cardiac effects

2.1. Lead

Lead (Pb) is a post-transition metallic element with a density of 11.35 g/cm^3 plus an atomic number (Z) of 82 and an atomic weight of 207 (though its atomic weight can vary due to the presence of several

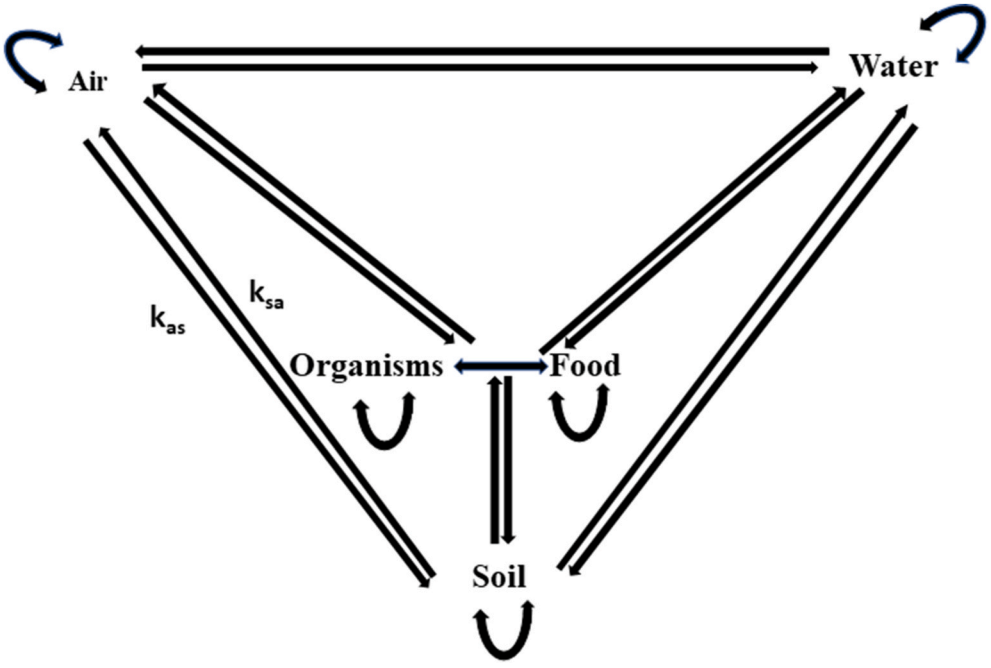

Fig. 1. Environmental analyses and their interrelationship in heavy metals' exposome. Heavy metals are persistently accumulated in the air, water, bioorganisms, and food in a particular ecosystem. Any epidemiological analysis must consider monitoring the heavy metal levels in these important ecosystems and also have an idea of the transformative reactions that can take place in this network that is in dynamic equilibrium. These transformative reactions at variance with chemical reactions are not fixed values depending instead on physicochemical conditions of the environment and other components characterizing the ecosystem in a particular space and time (for example in the figure they are noted as a particular example as ksa and kas, those are the transformative reactions between soil and air and air and soil respectively). Heavy metals are non-biodegradable, and they are distributed among the components of this dynamic network while accumulating over time. The arrows represent the passage from one media to another or living forms inside the ecosystem and vice-versa. Each medium can impact the central bioorganisms of an ecosystem while other organisms (Org) can exist as food. All the components are interrelated. The circular arrows represent several free elementals, cationic or organic forms of heavy metals that can interconvert between each other in a particular component of the ecosystem. The remediation of an exposome has to consider the elimination of the toxic forms of the heavy metals in the environment using, for example, powerful chelators that would diminish their toxicity.
Size Fig. 1: 1 column fitting image (also indicated in the file name).

isotopes)(Casas and Sordo, 2011). This heavy metal is the element with the highest atomic number among the non-radioactive elements, and it is also the final element in several pathways of radioactive decay (Watt, 2002). Its electronic orbitals are expressed as $[Xe]4f^{14}5d^{10}6s^{2}6p^{2}$. The electronic profile of Pb produces several consequences: *(a)* Lead can be easily oxidized to +2 or +4 ions by loss of two *p* or two *p* and 2 *s* electrons. The most commonly found form in nature is +2 (Pb^{+2}), except in carbon-like compounds. Pb^{+2} may participate in organic or inorganic red-ox pathways, affecting especially sulfhydryl and electron donor groups in biomolecules (Halmo and Nappe, 2021). This in turn causes reactive oxygen species (ROS) imbalance, eventually resulting in cell apoptosis, which can also occur due to the effects of other heavy metal ions, such as oxidized mercury (Hg^{2+}) (Almeida Lopes et al., 2016; Garza-Lombó et al., 2018; Jaishankar et al., 2014). *(b)* Because of its compact *f* and *d* orbitals Pb^{2+} has a relatively low ionic radius (112 p.m.), a charge-to-ionic radius ratio of 1.55, a cubic-structure and a coordination number quite similar to Ca^{2+} (Christensen, 2002; Slater, 1964). Due to its similarity to Ca^{2+} it has either agonist or antagonist effects in those biomolecules and biochemical pathways that require Ca^{2+}or other divalent cations to function (Dudev et al., 2018; Kirberger and Yang, 2008). Hence, Pb^{2+} exposome may interfere at several levels by various mechanisms, including Ca^{2+}-involvement in cell excitability, as a second messenger, and in Ca^{2+} homeostasis.(Dressier et al., 1999; Ferreira de Mattos et al., 2017; Kirberger et al., 2013; Kirberger and Yang, 2008; Mitra et al., 2017; Simons, 1993). Fig. 2 shows atomic lead, in its ionized form, and some likely mechanisms of damage in living organisms. .

Lead as a toxic substance for life was suspected since the second century BCE by the Greek physician Discorides (Needleman, 1989). The seminal work related to measuring the age of the earth by Claire Cameron Patterson in the late 1940s, also resulted in the recognition that industrial contamination by mankind was, in part, due to widespread Pb pollution (Landrigan, 2018; Nriagu, 1998). At the time leaded gasoline played a huge role in worldwide exposome to Pb. In most countries leaded gasoline has now been banished, though lead remains an important cause of heavy metal poisoning through soil and dust accumulation and due to tiny particles in some paints, tap water from old pipes, pottery, cosmetics, and car batteries, among other sources. After decreasing lead contamination after it was banned from gasoline, the worldwide levels of lead contamination have risen again due to discarded car and other batteries (Gottesfeld, 2017; Riva et al., 2012).

Lead contamination is an important health concern worldwide due to its systemic impact and promotion of several diseases. Indeed, Pb has been epidemiologically linked to the incidence of several specific diseases (Landrigan et al., 2002). Thus the Pb^{2+} exposome concept is the basis for a serious public health concern worldwide, especially when excess Pb^{2+} enters an organism during lead poisoning (Gidlow, 2015). Lead exposomes have to be identified, mapped, monitored, and remediated. This requires regular screening of this metal in children and sentinel living forms in lead-contaminated areas (Bischoff et al., 2010; Filippelli et al., 2018).

Lead poisoning can be acute or chronic (saturnism), and it can affect many organs and cells from conception to adulthood. Persons living in lead-polluted areas, especially children under 6 years of age, are at high risk of developing lead poisoning, which can affect childrens' development and promote irreversible health problems (Wani et al., 2015; WHO, 2019).

As we have mentioned before, there are no safe limits for lead concentrations in the blood. The statistical analyses have shown that blood levels above 5 μg/dL (~0.25 μM) in the blood of children must be considered unsafe and must be monitored, whereas a high risk of lead poisoning was generally found at higher concentrations, above 45 μg/dL (~2.25 μM) (Harper and Shannon, 2007). A direct extrapolation from the *in-vivo* blood level values to acute *ex-vivo* experimental values is not particularly accurate, because the solutions and experimental conditions are not identical. In addition, the lead concentrations *in-vivo* can be underestimated, since Pb^{2+} is bound to many cellular components while also circulating in the blood (Jin et al., 2008), as recently highlighted by the U.S. Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC)(Wani et al., 2015; WHO, 2019).

Importantly, very high Pb concentration can be fatal (FDA, 2017; Mason et al., 2019). The WHO Institute for Health Metrics and Evaluation estimated that in 2013 approximately one million deaths occurred worldwide due to lead poisoning (Organization, 2013). Approximately 535000 children in the U.S. have blood levels above 5 μg/dl (HEALTH, 2016).

Once inside a higher organism, lead can be absorbed by binding to red blood cells and later it is being distributed between bones (stable storage) and soft tissues (labile storage), such as liver, bone marrow, kidneys, brain and other tissues. A portion of the circulating Pb will not be absorbed and will be excreted. However, the absorbed Pb can accumulate and can be present for long periods of time. The percentages of this distribution are different in adults and children: almost 90% is stably stored in the bones of adults, whereas in children this decreases to 70% (Rădulescu and Lundgren, 2019). The non-absorbed, excreted fractions of lead found in the urine and stool are higher in adults than those found in children. The frequent hand-to-mouth behavior of young children also exposes them to lead more than adults by increasing their oral intake. These may be some of the possible reasons that explain the higher susceptibility and greater risk of lead poisoning in children (Halmo and Nappe, 2021; Hoppin et al., 1997; Mitra et al., 2017; Ziegler et al., 1978).

Lead poisoning does not result in highly specific symptomatology, and thus it can be hard to detect until dangerous quantities of lead have been accumulated(Halmo and Nappe, 2021). In children and newborns, Pb poisoning can result in neurological manifestations, especially impairing their cognitive, behavioral, and physical abilities. Lead exposome before birth increases the risk for premature delivery, low weight at birth, and slow growth (Zhang et al., 2015). Lead-exposed children also have developmental delays and display learning difficulties, irritability, fatigue, and hearing loss (Leviton et al., 1993). These symptoms are likely due to an interference in synaptic communication mediated by lead exposome at the level of the central nervous system and this is one of the hallmarks of lead poisoning (Lidsky and Schneider, 2003).

In severe cases of acute encephalopathy caused by lead poisoning, seizures and coma can be observed, even in adults, due to damage of the cerebral microvasculature, edema, and an increase in intracranial pressure (de Souza et al., 2013). In adults, peripheral neuropathy is more frequently observed, although the underlying mechanisms are poorly understood (Thomson and Parry, 2006). Non-specific symptoms related to alterations in the central nervous system in adults are commonly found in lead poisoning, such as headaches, short-term memory losses, concentration problems, and mood disorders (Mason et al., 2014).The neurological perturbations can also be associated with alterations and symptoms of the gastrointestinal tract, such as anorexia, abdominal pain, constipation, vomiting, and weight loss. In children, there is also the additional tendency to eat things from the soil that aren't food [pica] (Mitra et al., 2017). The kidneys are also affected by lead exposure that can promote global proximal tubule dysfunction, competition for excretion of uric acid in the distal tubule, and formation of urate crystals in urine and joints similar to a gout-like condition(Barbier et al., 2005).

The endocrine system is another target of the Pb exposome inhibiting the growth and development of skeletal muscle, problems with reproduction, and function of the thyroid gland (Doumouchtsis et al., 2009). Hematological symptomatology can occur (as anemia) due in part, to lead effects on red blood cells through disturbances in pathways related to heme synthesis (for hemoglobin) and rupture of the plasma membrane causing hemolysis (Flora et al., 2012; Mitra et al., 2017).

Lead poisoning also influences the cardiovascular system causing hypertension by affecting multiple targets that control normal blood pressure through blood vessel flexibility, the renin-angiotensin-

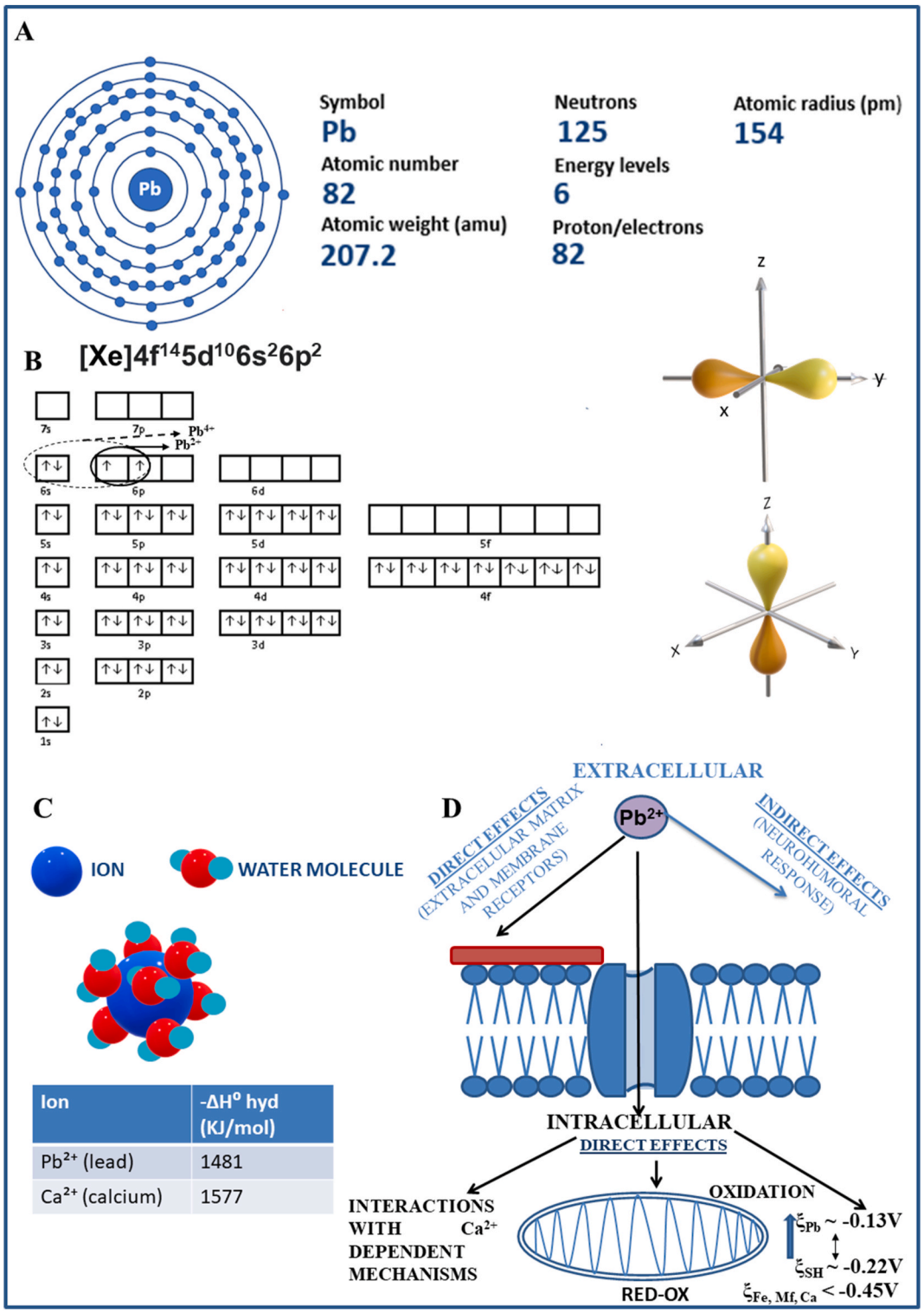

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Fig. 2. Chemical structure and general mechanisms of toxicity of lead (Pb). (A) A lead atom with its electron configuration (*left panel*) and its identification numbers as a chemical element (*right panel*). The outer layer of electrons contains 4 electrons located at the 6th energy level. (B) Energy levels of electrons in a Pb atom and outer unpaired electron orbitals (*px* and *py*). In the outer layer of electrons at the 6th level, the four electrons are two paired as *s* orbitals and two non-paired as *px* and *py* orbitals. Thus, it requires less energy to remove two electrons to become Pb^{2+}, than to remove the 4 outer electrons to yield Pb^{4+}, and as a result, the +2 forms of lead are more abundant in nature than the +4 forms. The ovals with a solid line and short dash line enclose the electrons lost to form either Pb^{2+} or Pb^{4+}. (C) Hydrated Pb^{2+} in water and its enthalpy hydration energy. Hydrated Pb^{2+} tends to adopt a cubic-like structure similar to Ca^{2+}. Each hydrated ion is surrounded by approximately 8 molecules of water with its oxygen atoms facing the positive inner divalent cation. Because of the similar size and hydration behavior of Ca^{2+} and Pb^{2+}, their enthalpy energies for dehydration from these cubic layers, are also similar. This explains why Pb^{2+} can interfere or interact directly with many sites that are modulated by intracellular Ca^{2+}. (D) Extracellular Pb^{2+} can promote changes in cardiac function by indirectly affecting the neurohumoral response that modulates heart function, and it can also exert local direct damage by interacting with the immediate extracellular matrix that surrounds cardiomyocytes, or by binding to receptors in the plasma membrane of these cells. Here, we present evidence that another mechanism of damaging action of the Pb^{2+} exposome is a direct mechanism resulting from Pb^{2+} entry into cardiomyocytes. After cellular entry they can interfere with all the mechanisms modulated or mediated by intracellular Ca^{2+} and also interfere with red-ox mechanisms due to its red-ox potential of approximately – 0.13 V. Cations like Fe^{2+}, Mg^{2+} and Ca^{2+} and also biomolecules in mitochondria and cytoplasm that contain organic groups with more negative redox potentials (like –SH groups in cysteines) can be oxidized by Pb^{2+}, producing oxidative stress.
Size Fig. 2: 2 column fitting image (also indicated in the file name).. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

aldosterone system, and autonomic neuropathy (Hu et al., 1996; Mitra et al., 2017). Though not as apparent as neurological symptomatologies, the heart is another target of the lead exposome and Pb poisoning (Alissa and Ferns, 2011; Ferreira de Mattos et al., 2017; Landrigan, 2018; Navas-Acien et al., 2007; Sachdeva et al., 2018).

2.2. Mercury

Mercury (Hg), has a density of 13.53 g/cm^{3}and it can poison people and the environment via its exposome. Hg has an atomic number (Z) of 80 and an atomic weight of 200 (though it has several isotopes). Its electron configuration is [Xe] $4f^{14}$ 5 d^{10} $6s^2$. It has a rhombohedral crystal structure with some resemblance to the cubic structures of other metals. It is the only liquid metal at room temperature and pressure. Hg has a stable electron configuration resulting in the formation of weak bonds with other molecules or mercury atoms. Because of this, elemental mercury (Hg^0), can melt at room temperature and form a liquid. Several chemical forms of Hg can be found in a mercury exposome, and the different forms can result in different types of mercury poisoning, though all of the chemical forms of mercury are capable of interconverting with each other (Wise, 2016). The sources of Hg can be natural (volcanic eruptions) or anthropogenic (mining, paintings, hypochlorite production, mercury lamps, and others). Most of the Hg exposomes are the result of increased anthropogenic sources and these are increasing on the earth's surface with time to become a public health issue, especially in underdeveloped countries (Genchi et al., 2017).

As with Pb^{2+}, there is no safe level of Hg exposure. When Hg is found in organisms, the implication is that Hg has or is in the process of developing its toxic effects. However, the symptoms of Hg toxicity may not appear in humans until the levels of Hg in urine are above 50 μg/L. Hg can be fatal when urine concentrations are higher than 800 μg/L (Ye et al., 2016). As with Pb^{2+}, the correspondence of Hg amounts between *in-vivo* and *ex-vivo* results is fraught with errors because of the differences found in various solutions and bioavailability, plus a tendency to underestimate the values measured *in-vivo* (Sherlock and Quinn, 1988).

Hg in exposomes is found as inorganic or organic forms. Inorganic mercury can be divided into elemental mercury (Hg^0) and mercury salts (mercurius or Hg^+ and mercuric or Hg^{2+}salts) (Sakamoto et al., 2018; Yang et al., 2020). Hg^+ and Hg^{2+} have an atomic radius between 130 and 150 p.m. (Aylett, 1985), although the most common form in mercuric salts is Hg^{2+}. These two oxidative forms of Hg can interconvert between each other, and their toxic effects on ecosystems and people are different. If Hg is oxidized (especially after exposure to acids), its most common form is as a +2 cation (Hg^{2+}). This +2 (mercuric) cation forms tetrahedral complexes with certain anions like chloride ($HgCl_2$), though it can also react with oxygen and ammonia (important interactions for its mechanism of toxicity) (Aylett, 1985). Some of these mercuric salts are easily dissolved in water, generating important mercury exposomes that can enter the Hg organism mostly by absorption through the gastrointestinal tract (Rani et al., 2019).

The identification of Hg poisoning can be difficult, because its exposome generates multisystemic, non-specific symptoms, including shortness of breath or respiratory distress, headache, ataxia, dementia, visual problems, metallic taste, and abdominal gastritis-like pain, whose relative intensities can vary according to the presentations of Hg (Rani et al., 2019). The elimination of Hg from the body takes place through urine excretion or through the gastrointestinal tract with half-lives of up to 70 days (Sauder et al., 1988). The gastrointestinal mucosa and proximal renal tubules are damaged by Hg through oxidative stress and also by the impact of Hg on those host molecules that require ions of similar size as a cofactor.

Hg can also form highly toxic organic mercury compounds with short or long-chain carbon molecules, that do not react with water and can penetrate into cells. Moreover, Hg does cross the blood-brain barrier, accumulating and promoting damage in the nervous system when converted to inorganic Hg (Rani et al., 2019). The most common form is methylmercury (MeHg) (Clarkson and Magos, 2006; Compeau and Bartha, 1985; Guzzi et al., 2006; King et al., 2000). The exposome to organic mercury often takes place after ingesting contaminated seafood and fishes, as well as Hg-containing paints (Sakamoto et al., 2018).

Elemental mercury (Hg^0) is highly volatile, and in the atmosphere it can be found as a gas. Its exposome can result from Hg in thermometers, lamps, hypochlorite production, and disinfection (Zhao and Rochelle, 1999). Hg^0 enters organisms by inhalation or by ingestion, and it can cross the blood-brain barrier and form deposits in the central nervous system.

Hg promotes damage by binding to sulfhydryl (SH), amide, and carboxyl groups in proteins, by affecting selenium-binding proteins that are important as antioxidants, and it interacts with phosphoryl groups in biomolecules resulting in dysfunction of enzymes, transport, and structural proteins (Clarkson and Magos, 2006; Spiller, 2018; Syversen and Kaur, 2012; Yang et al., 2020). Hg alters cysteine-containing SH groups, known to be precursors of antioxidant molecules like glutathione (Quig, 1998). Hg^{2+} might produce damage by interfering with Mg^{2+}-binding sites because of their similar enthalpy energy dehydration values (Beavis, 1991; Guiet-Bara et al., 1991). Fig. 3 summarizes the chemistry of mercury, the forms of presentation of Hg and its proposed mechanisms of damage.

Hg has been used in mining processes and in farming as a pesticide; thus it can produce complex exposomes affecting potentially millions of people. Significant Hg contamination has occurred during mining, such as in the Minamata Bay mining incident and in the Agano River contamination in Niigata, Japan and during farming pesticide incidents in Iraq incidents (Bakir et al., 1973; Harada, 1995). These are well-known dramatic examples of community contamination by Hg.

Individually, children and fetuses in pregnant women are highly

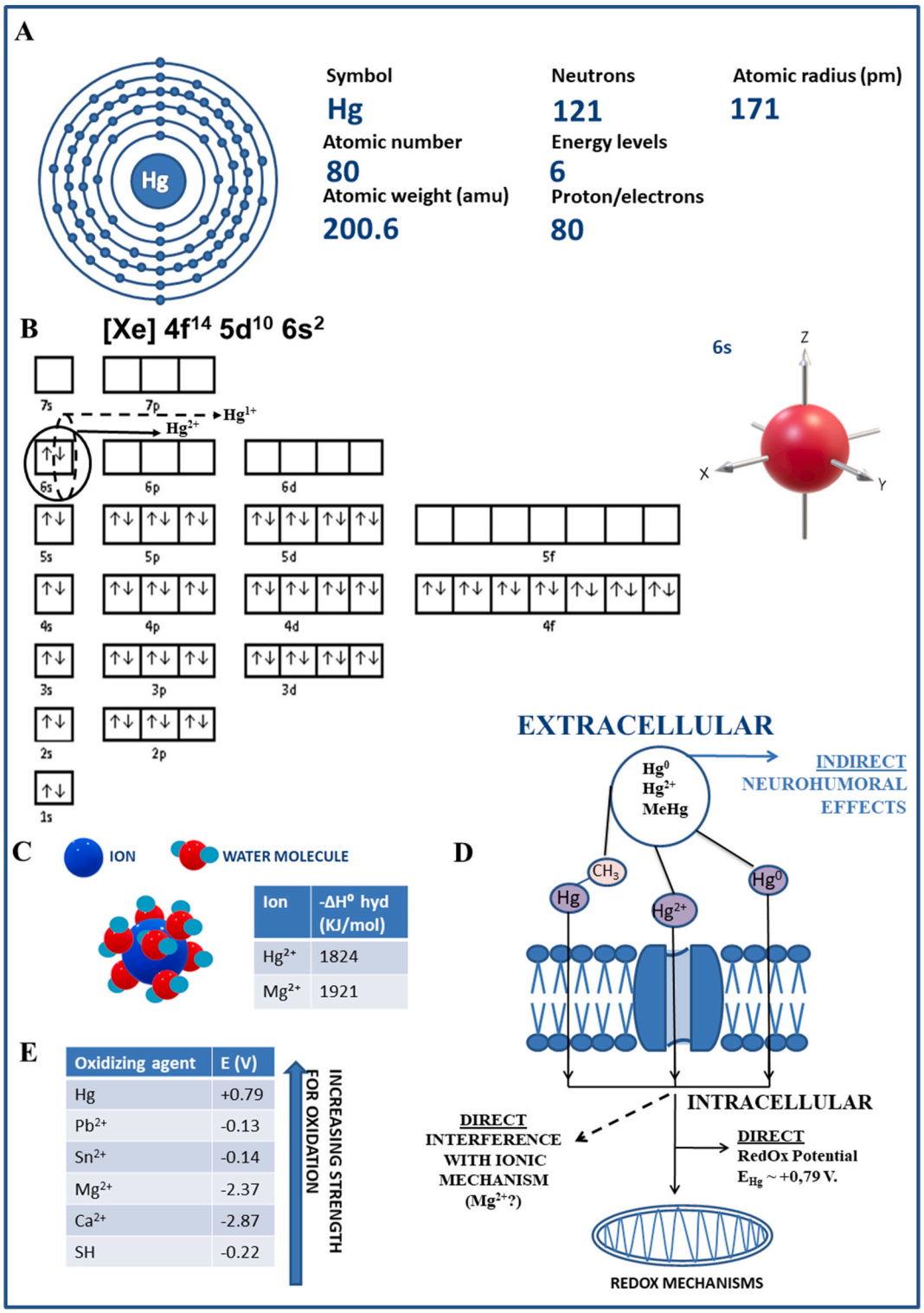

Fig. 3. Chemical structure and general mechanisms of toxicity of mercury (Hg). (A) Main chemical features of mercury as it was done for Pb^{2+} (Fig. 2A). The outer layer of electrons contains 2 electrons located at the 6th energy level. (B) Electron energy levels and outer paired electron orbitals (*s*) for Hg as it was analyzed for Pb^{2+} (Fig. 2B). In the outer layer of electrons at the 6th level, the two electrons are paired as *s* orbitals of different ½ spins. Thus, it is more likely that Hg will lose two electrons to form Hg^{2+}, than to remove only 1 outer electron to form Hg^{1+}. As a result, the +2 forms of mercury (mercuric salts), are more abundant in nature than the +1 forms (mercurious salts). The ovals with a solid line and short dash line enclose the electrons lost to form either Hg^{2+} or Hg^{1+}. (C) Hydrated Hg^{2+} in water and enthalpy hydration energy are similar between Mg^{2+} and Hg^{2+}. Thus Hg^{2+} might interfere and perhaps interact directly with sites modulated by intracellular Mg^{2+}. (D) Extracellular Hg can be present as inorganic salts (Hg^{2+}), elemental mercury (Hg^{0}), and organic forms such as methyl-Mercury (MeHg). The ionized forms of Hg require membrane transport mechanisms not yet identified. After entering cardiomyocytes, Hg^{2+} might directly interfere with mechanisms modulated by intracellular Mg^{2+}. However, its main mechanism of damage is oxidative stress. (E) Redox potentials. Due to its redox potential ($\xi_{Hg} \sim +0.79$ V), Hg promotes generalized oxidation of biomolecules and intracellular cations.
Size Fig. 3: 2 column fitting image (also indicated in the file name).

susceptible to mercury exposome; thus special precautions against the ingestion of contaminated foods, such as fish, should be undertaken with children and pregnant women in areas of high risk of Hg contamination (Rani et al., 2019). Elemental Hg is also a health risk and can affect the lungs, central nervous system, kidneys, and cardiovascular system, and the salts of Hg cause irritation of the gastrointestinal tract. Organic mercury can accumulate s in the central nervous system for years, causing developmental neurological symptoms (Rani et al., 2019). Also, elemental Hg in the bloodstream can be rapidly oxidized into Hg^{+} and Hg^{2+} by catalase and peroxidase, and this can alter-SH groups found in cysteines, and impair the cellular antioxidant protection mechanisms (Ballatori et al., 1985). All forms of Hg can produce cardiovascular effects (this will be discussed in more detail in section 4).

2.3. Experimental approaches to study the direct effects of lead and mercury in cardiac function

The direct effects of lead (and mercury), on heart excitability reported in this review, were explored using physiological extracellular Tyrode 1.8 mM Ca^{2+} solutions with different concentrations of PbCl2 (or $HgCl_2$) added (described in (Costa et al., 2014), and (Ferreira de Mattos et al., 2017). In these experimental conditions, isolated hearts or isolated cardiomyocytes, were exposed to μM levels of Pb^{2+} or Hg^{2+}. The intracellular solutions used under whole-cell patch clamp in isolated cardiomyocytes were in mM: 140 CsCl, 2 $MgCl_2$, 5 EGTA, 10 HEPES, and 5 ATPMg. Signals were recorded with 1–3 MΩ pipettes and an Axopatch 200B Patch Amplifier (Molecular Devices, San José, Californa, USA) at 1–5 KHz. To record intracellular Ca^{2+} (or Pb^{2+}), permeant Ca^{2+} sensitive dyes (or Pb^{2+} sensitive), were added to the extracellular solutions as reported in the Molecular Devices user manual for fluo-3AM for Ca^{2+}(or Calbryte, and Leadmium-Green AM, LG-AM, the later for Pb^{2+}) (Molecular Devices, San José, Californa, USA). Excitation was performed at 488 nm and emission collected at 510 nm using a Leica Sp5 Confocal Microscope joined to the patch setup. To avoid interferences from these heavy metals with the Ca^{2+} sensitive dyes, relative variations in length upon extracellular stimulation were recorded using transillumination

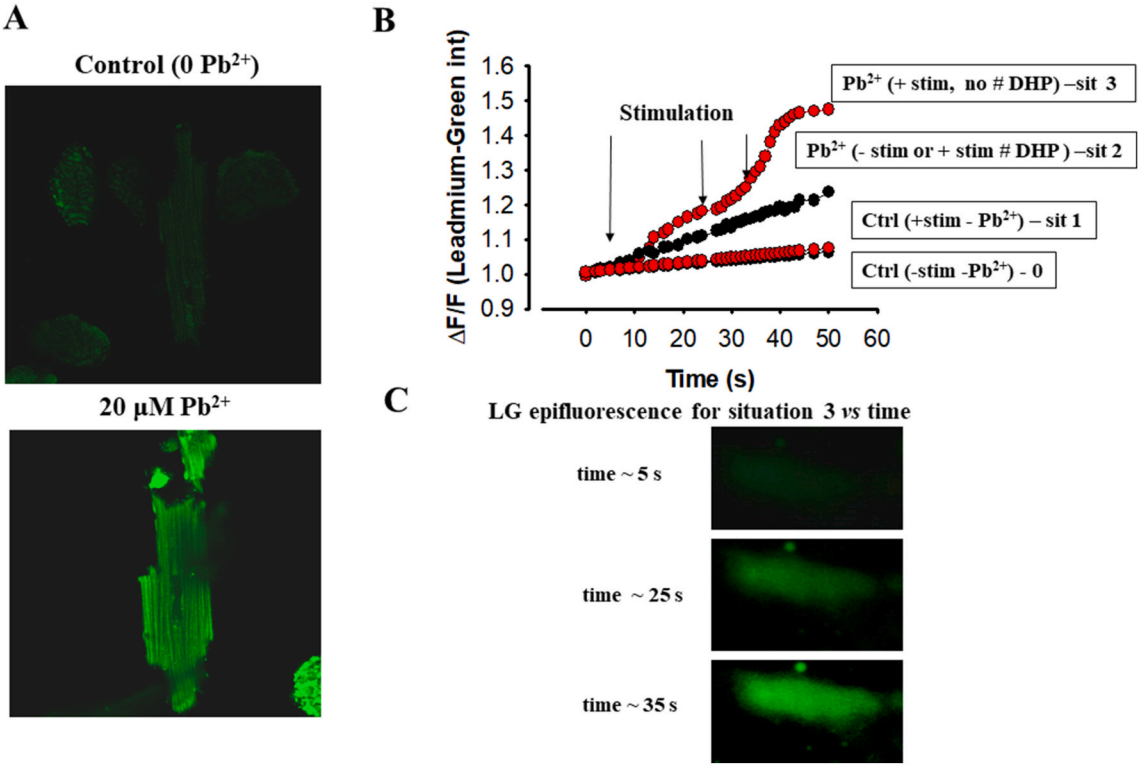

Fig. 4. Lead entry into cardiomyocytes involves Cav1.2 channels. (A) Cardiomyocytes loaded with leadmium-green (LG) observed in a confocal microscope, a specific dye that fluoresces upon Pb^{2+} binding. The cells do not show fluorescence in control physiological solutions containing 1.8 mM extracellular Ca^{2+} (*top*), but cells became fluorescent when 20 μM Pb^{2+} was added to the extracellular solution (*bottom*). This experiment is necessary to assure that reported signals arise from the Pb^{2+} binding present inside the cells. Images were taken with a Leica SP5 confocal microscope. (B) Plot of fluorescence from cardiomyocytes loaded with LG *versus* time in different situations. Situations 0 and 1 were used as controls. In situation 0 loaded cardiomyocytes were placed in a control medium without Pb^{2+} and also non-field stimulated to elicit action potentials. In situation 1, the experiment from situation 0 was repeated with field stimulation. In both cases, the fluorescence slightly increased linearly over time indicating that there was no significant increment of fluorescence. In conclusion, if Pb^{2+} is not present in the extracellular solution there is a non-significant increase of fluorescence with time (situations 1 and 0). In situation 2, 20 μM $Pb^{2+}$was added to the extracellular solution in the absence of stimulation. The addition of Pb^{2+} made the slope of the straight-line steeper, suggesting that part of the entry of Pb^{2+} into cardiomyocytes is due to non-voltage-dependent mechanisms or transport pathways. Without doing patch-clamp and performing trains of field stimulation that elicit action potentials, cardiomyocytes pre-loaded with LG and extracellular Pb^{2} become more fluorescent as the time of exposure to Pb^{2+} goes on. When action potentials are elicited, currents through Cav1.2 channels are present. In situation 3, 20 μM $Pb^{2+}$was added to the extracellular solution in the presence of field stimulation to elicit action potentials (*arrows*). Each arrow represents times of application of train pulses to elicit action potentials. Fluorescence increases immediately after their application. The linearity of the fluorescence over time disappeared and it was hastened by the presence of field-stimulation (*arrows*). If 2 μM dihydropyridine (DHP) was added to the medium, the course of fluorescence increase over time was linear as in situation 2 (non-field stimulated). These results suggest the involvement of Cav1.2 channels in the entry of Pb^{2+} into cardiomyocytes. (n = 5). (C) Epifluorescence images of cardiomyocytes in situation 3, immediately after being field-stimulated by a train of pulses able to elicit action potentials at different times (5, 25, and 35 s). Pulses were applied to cells loaded with LG placed in a media containing extracellular Pb^{2+}. Experiments were performed in an Olympus IX-81 epifluorescence microscope. (Unpublished results, *in preparation*).
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with the confocal microscope. These procedures were the same for the experiments reported from Figs. 4–6 and the experiments performed to obtained the data shown in Tables 1A and 1B. Some are different experiments from previously published work and other results shown are not published, though they were presented at scientific meetings and symposia and they are currently *in preparation*.

3. Cardiac effects of the lead exposome

The cardiac effects of the lead exposome are especially important, because they have been underestimated and understudied in comparison with other health effects of the Pb^{2+} exposome. Studies on the effects of Pb^{2+} on the heart *in-vivo* can yield different results compared to those observed *ex-vivo* or *in-vitro,* because the heart is intensely modulated by autonomic innervation and humoral responses. For example, Pb can affect cardiac function by dysregulation of humoral and neuronal

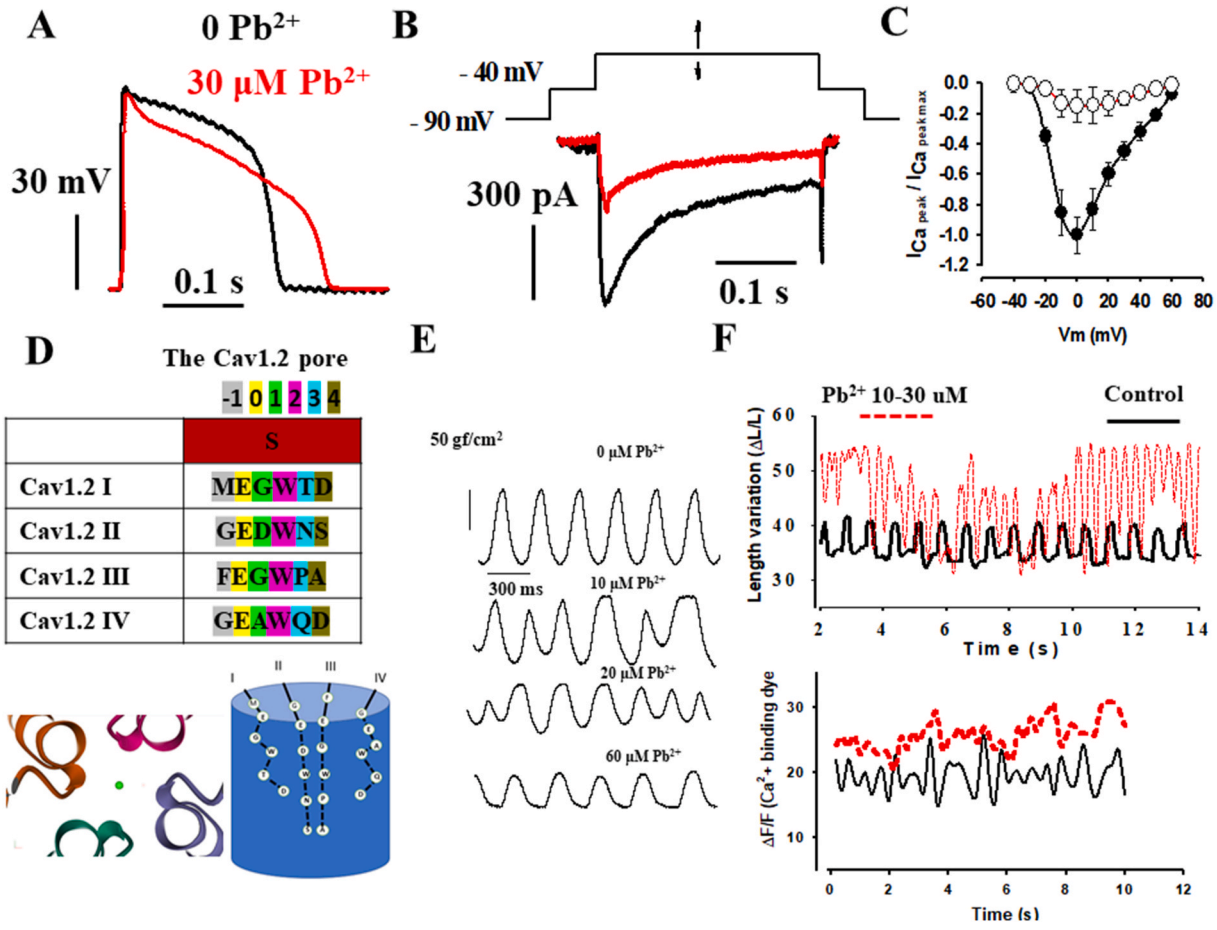

Fig. 5. Lead has direct effects in cardiac excitability and contractility. (A) Action potentials measured in isolated cardiomyocytes in control medium (*black*) and in medium containing 30 µM extracellular Pb^{2+}(*red*). The presence of extracellular Pb^{2+} increases the action potential duration with little reduction of the voltage at the plateau or phase 2 stage. (B) Cav1.2 currents (ICa) elicited by the pulse protocol shown above the currents. The addition of extracellular Pb^{2+} reduces the size of the currents, while it increases their fast inactivation but reduces the slow inactivation. As a result, the currents last long times at low values (n = 5) (C) Current-voltage relationship of ICa without (*black symbols*) or with extracellular Pb^{2+}(*white symbols*). The addition of 30 µM Pb^{2+} interferes with ICa at all voltages, (n = 3). (D) Putative structure of the Cav1.2 pore domain. The structure of this domain is critical to understanding Pb^{2+} substitution of Ca^{2+} at this level. On *top*, the alignment of the selectivity filter of the channel for each of the four repeats (1 to IV) is shown. The glutamates (***E***) play a key role in the coordination with positive charges from divalent cations permeant through the channel. Below is shown structural data and a scheme of the 3D arrangement of the pore. As the ionic radius and enthalpy energies for dehydration are quite similar between Ca^{2+} and Pb^{2+}, Pb^{2+} would fit in the conduction pathway of Cav1,2 and enter into cardiomyocytes through this channel. The putative structure shown was previously reported (Abderemane-Ali et al., 2019). (E) Tension records *versus* dose of exposure to Pb^{2+}in isolated hearts. At low Pb^{2+}concentrations uncoordinated contractions with the heart losing a regular rhythm. (F) Measurement of contractions and calcium transients elicited by repetitive field stimulation as length variations in control medium and in medium containing 10–30 µM Pb^{2+} in isolated cardiomyocytes. Surrounding the cardiomyocytes are electrodes that were connected to a pulse generator that was used to apply repetitive stimulation in the field. After each action potential was triggered by the pulses, the myocytes shorten and release Ca^{2+}. Both, the relative perpendicular length along their sarcomeres (ΔL/L) and relative change in fluorescence after Ca^{2+}-binding to a Ca^{2+} sensitive dye (ΔF/F), could be measured. At the *top* the relative variation in length in a direction perpendicular to the sarcomeres is plotted *vs.* time for both conditions. In a physiological control medium solution, the repetitive stimulation promoted regular contractions over time (*solid black line*). In a solution containing 10–30 µM $Pb^{2+}$the pattern of contraction promoted by each pulse was highly variable and non-synchronized in space and time in the cardiomyocyte (*short-dash red line*). At the *bottom*, the same experiment was repeated measuring intracellular Ca^{2+} with a Ca^{2+} sensitive dye with low affinity for Pb^{2+}(Calbryte). The calcium transients were observed in a more regular pattern in control physiological solutions (*solid black line*), than obtained after exposure to 10–30 µM Pb^{2+}. In the latter situation basal Ca^{2+} was increased in comparison with the control and the variations of Ca^{2+} concentrations inside the cell did not seem to be coordinated with the repetitive stimulus. The results suggest a dysregulation of intracellular Ca^{2+} homeostasis mediated by Pb^{2+} through its blockage of Cav1.2 channels and entry into the cardiomyocytes. (Unpublished results, *in preparation*).
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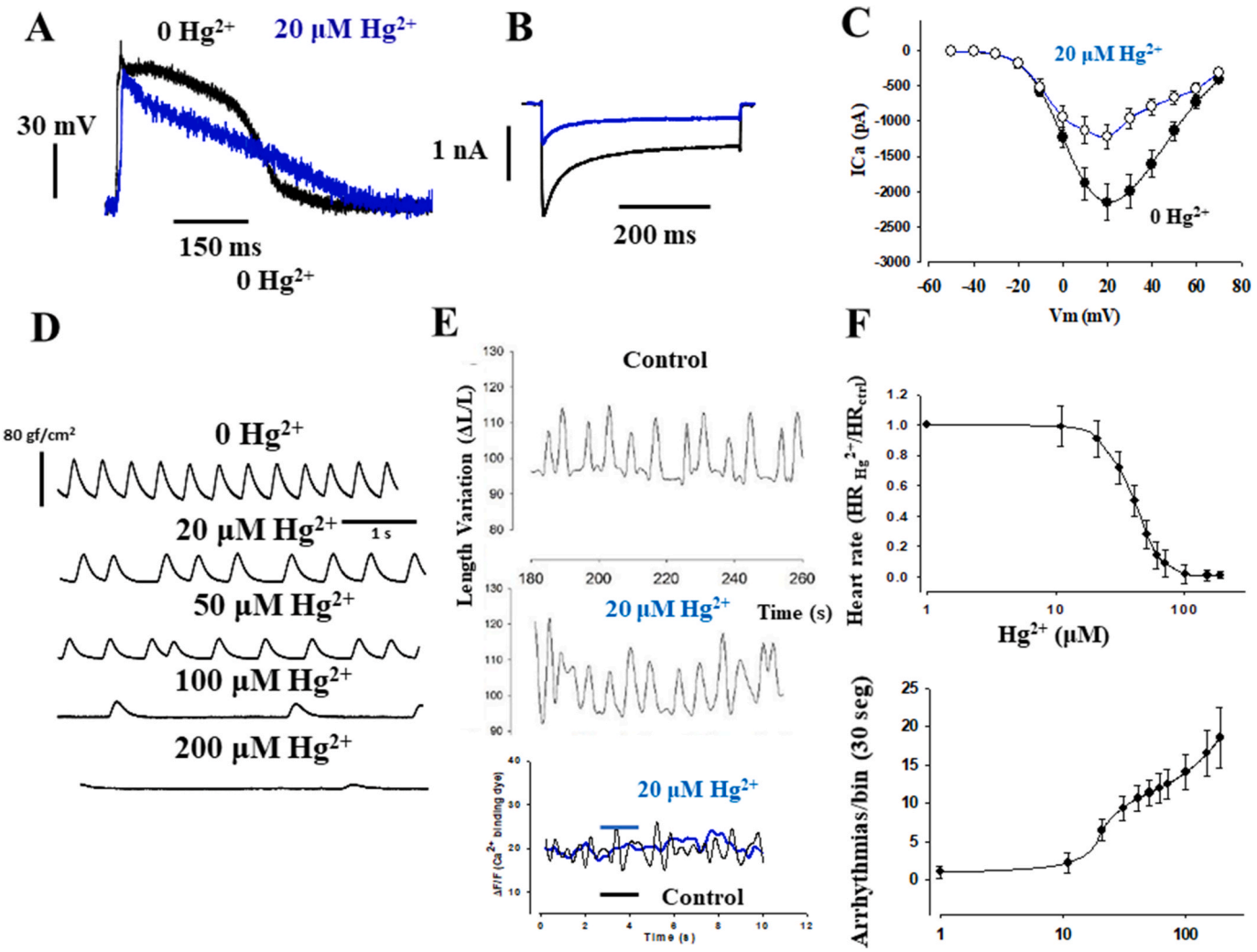

Fig. 6. Cardiac excitability and contractility are directly affected by Mercury. (A) Action potentials in isolated cardiomyocytes (control (*black*) and 20 µM extracellular Hg^{2+}(*blue*) as in Fig. 5A for Pb^{2+}. Extracellular Hg^{2+} increases the action potential duration suggesting an inhibition of Hg^{2+} of the delayed rectifier K^+ channels, in addition to the Cav1.2 channel blockage. (B) ICa L blockage by Hg^{2+} (control medium (*black*) and upon addition of 20 µM extracellular Hg^{2+}(*blue*) as in Fig. 5B for Pb^{2+}. (C) Current-voltage curve (control (*black*) and in medium containing 20 µM extracellular Hg^{2+}(*blue*), as in Fig. 5C for Pb^{2+}). (D) Hg^2 has a direct negative inotropic effect in isolated hearts as shown for Pb^{2+} in Fig. 5D. A pronounced negative chronotropic effect is also seen as the heart rate diminishes with increasing Hg^{2+} concentration. (E) Measurements of single cell contractions and intracellular Ca^{2+} (control (*black*) and with 20 µM extracelular Hg^{2+} (*blue*), as in Fig. 5E for Pb^{2+}). Single cell contractions were obtained as described in Fig, 5E for Pb^{2+}. (F) Relative length variation promoted by 20 µM Hg^{2+}, as it was analyzed for Pb^{2+} in Fig. 5F. At the *bottom*, the experimental results for length and intracellular Ca^{2+} are shown. Both, contractions and Ca^{2+} transients were observed in a more regular pattern in control physiological solutions (*solid black line*), than after exposure to 20 µM Hg^{2+} (*short-dashed blue line*). The results suggest a dysregulation of intracellular Ca^{2+} homeostasis promoted by Hg^{2+} that is different than that observed with Pb^{2+}, suggesting different mechanisms of promoting negative inotropism by these heavy metals. (F) Mercury promotes negative chronotropism and arrhythmias. The dose-response curve of the normalized heart rate *versus* dose of exposure to Hg^{2+}in isolated hearts is shown in the *left panel*. The solid line represents the best fit of a Hill equation and shows the tension records of the isolated hearts. IC50 is approximately 50 µM. The dose-response curve of arrhythmic events per 30s bin *versus* dose of exposure to Hg^{2+}in isolated hearts is shown in the *right panel*. Increasing concentrations of Hg^{2+} are accompanied by an increasing number of arrhythmic events per bin. (n = 5, both plots). (Unpublished results, *in preparation*). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

modulation, but it can also affect ardiac vessels, innervation, and by entering into the cardiomyocytes, it can interfere with red-ox mechanisms and calcium signaling and homeostasis.

Since the 1960s, myocarditis has been identified in children exposed to Pb^{2+} (Freeman, 1965). While in older adults most of the cardiac effects of lead have been associated with promotion of atherosclerosis or secondary effects due to hypertension in people living in exposome lead areas (Kurppa et al., 1984). Measurements of lead exposome damage have been related to mortality, histopathological changes in the myocardium, and heart failure. These have been extensively reported by Kopp and Tollestrup (Kopp et al., 1988; Tollestrup et al., 1995), reviewed by Bhatnagar (2006), and Navas (Navas-Acien et al., 2007). Such studies concluded that the damage to the heart by Pb^{2+} exposomes was more complex than initially thought.

A clear outline from previous works is that now is accepted that even low-level Pb^{2+} exposomes increase cardiovascular risk and that not all of the cardiac damage produced by lead is secondary to the promotion of other diseases by the Pb^{2+} exposome (Skoczynska and Skoczynsk, 2012; Williams et al., 1983). The agonistic binding of Pb^{2+} and Ca^{2+} to the myosin light chains in smooth muscles surrounding cardiac vessels may be important in explaining why hypertension is often linked to people living in Pb^{2+} exposomes (Chao et al., 1995).

In addition to affect the blood vessels causing hypertension, another picture that emerged from published studies have directly linked the lead exposome to cardiac dysfunction. For example, echocardiographic data in humans demonstrated that the Pb^{2+} exposome impairs left

ventricle function (Yang et al., 2017). Hypertension promoted by Pb^{2+} can affect the heart by work overload promoting heart failure (Chao et al., 1995). Regarding contractility, experiments exposing isolated hearts from rats to extracellular Pb^{2+} showed a negative inotropic effect of Pb^{2+}exposure (Carmignani et al., 2000; Prentice and Kopp, 1985; Vassallo et al., 2008). These experiments are consistent with the negative inotropic effect found after chronic exposure to Pb^{2+} by breastfeeding of neonatal rats (Williams et al., 1983). It has been proposed from the experiments in rats that even low levels of Pb^{2+} interfere with the contractile proteins of the heart and their contraction cycle (Katsnelson et al., 2020; Silva et al., 2015). More recent reports also found a negative inotropic of the Pb^{2+} exposome (Klinova et al., 2020; Protsenko et al., 2018, 2019). However, some reports maintain that Pb^{2+}has a positive inotropic effect in rats (Fioresi et al., 2014). Thus, this remains a controversial issue that may depend on the different experimental conditions used by each research group, though most of the reports are consistent with a negative inotropic effect of the Pb^{2+} exposome. In *ex-vivo* experiments with isolated hearts or cells from guinea-pigs, there was a negative inotropic effect (IC50 ~ 80 μM) consistently with the reports in rats (Silva et al., 2015; Vassallo et al., 2008; Williams et al., 1983). Changes in heart excitability have been associated with the levels of the Pb^{2+} exposome. Correlations have also been established between Pb^{2+} exposomes and heart automatism, and conduction observed in electrocardiograms (ECG) of populations living or working in certain regions with increased risk of Pb^{2+} exposure (Jing et al., 2019; Kiełtucki et al., 2017). These latter studies report a Pb^{2+}-mediated prolongation in the QT interval (Chen et al., 2013 Kiełtucki et al., 2017) and alterations in the QRS-T angle (Jing et al., 2019). Both events are associated with arrhythmias and sudden cardiac death (Anderson, 2006; Bergfeldt et al., 2020; May et al., 2017; Schwartz and Wolf, 1978). Prolonged conduction between atria and ventricle (P-R interval prolongation) has also been reported, among other conduction disturbances that can develop into cardiac arrhythmias (Cheng et al., 1998; Myerson and Eisenhauer, 1963). A related phenomenon, the rates of arrhythmias documented in workers with occupational exposures to Pb^{2+} has been estimated, and it is considerably higher than in unexposed control groups (Karakulak et al., 2017). The autonomous innervation of the heart is also affected by Pb^{2+}, hence changes in heart rate variability have also been found in employees working under conditions of Pb^{2+} exposome (Andrzejak et al., 2004; Madan et al., 2007). In addition to the humoral and innervation damage promoted by Pb^{2+}, this heavy metal may also impact HCN channels, critical for the autonomic triggering of action potentials in the sinoatrial node in the heart. It has been determined that Pb^{2+} blocks Cav1.2 channels, but it does carry currents for a prolonged time in ventricular and atrial cells, increasing the risk for arrhythmic events in ventricles and atria (Ferreira de Mattos et al., 2017; Tobón et al., 2017). Experiments performed *in-vivo* in neonatal rats show that breastfeeding neonatal rats with lead in the milk, enhanced approximately four times the susceptibility of neonates to arrhythmias (Williams et al., 1983).

Some reports have addressed the effects of *ex-vivo* lead exposure in isolated hearts and cardiomyocytes. Among other insights, these experiments have been useful in determining how lead can enter cells, and in particular, they have provided insights into the molecular mechanisms of lead exposome damage to heart cells. Using fluorescence compounds like Leadmium-green (LG), a specific Pb^{2+}-binding dye that increases its intensity of fluorescence when Pb^{2+} is bound, the uptake or entry and distribution of Pb^{2+} in cardiomyocytes or other cells can be directly observed (Singh et al., 2019). Fig. 4 shows in cardiac cells isolated from guinea-pigs that Pb^{2+}, due to its similarity to Ca^{2+}, can enter these cells through Cav1.2 L-type Ca^{2+} channels (at least partly) when in the micromolar range of concentration, agreeing with previous reports (Ferreira de Mattos et al., 2017). It also shows that Pb^{2+} could enter cells through non-voltage-dependent pathways. For example, Trp channels with Ca^{2+}-permeant pores are known to be secondary entry pathways for divalent cations, in addition to other transport proteins like the Na^{+}-Ca^{2+} exchanger (NCX), SOC channels, Zn^{2+} and divalent metal transporters (DMT), pH-sensitive transporters, and aquaporin channels (Amado et al., 2012; Bressler et al., 2004; Cheong et al., 2004; Fu et al., 2014; Liu et al., 2007). Since these ion transport systems are widely distributed throughout various tissues, the experiments in isolated hearts and cells, are useful helping to explain the multisystemic effects of lead exposome on various cells, tissues and organs.

An intriguing aspect of the lead exposome that remains unclear is that extracellular Pb^{2+} may exert some of its effects through binding to the abundant membrane receptors displayed on the external cell surfaces of cardiomyocytes and other cell types. Also, it is not clear if Pb^{2+} can affect cardiac function and cell function in general by interfering with the cardiomyocyte extracellular matrix. For example, Pb^{2+} could replace Ca^{2+} in the extracellular matrix and interfere with Ca^{2+} mediated extracellular processes.

Once Pb^{2+} enters cardiomyocytes, it can promote changes in redox potential and calcium signaling (Almeida Lopes et al., 2016; Kirberger et al., 2013). These changes become evident as alterations in basic functions of the heart, such as excitability, automatism, heart rhythm, and contractility. Ventricular action potentials in cells exposed to Pb^{2+} are prolonged when compared with non-exposed control cells (Fig. 5A). The results in Fig. 5A are consistent with those reported previously on monophasic action potentials (Ferreira de Mattos et al., 2017). The findings also imply a blocking action of Pb^{2+} on the delayed rectifier K^{+} channels (KCNQ and hERG). This seems quite likely, since hERG has been reported to be blocked by extracellular Ca^{2+} (Nguyen et al., 2015). A persistent current through Cav1.2 channels combined with hERG blockage could explain the observation of prolongation of the action potentials and QT intervals. This result is consistent with clinical observations in human populations living in Pb^{2+} exposome areas, because the QT interval prolongation observed in the ECG can be explained by a longer duration of phase 2 or plateau phase of the cardiac action potentials (Ponte et al., 2009). The prolongation of the cardiac action potential can be explained by longer persistence of the currents carried by Pb^{2+} entry through these channels, even though the amplitude of peak Cav1.2 currents is diminished (Fig. 5B). These currents are carried by Pb^{2+} and Ca^{2+} through the Cav1.2 channels, but they are smaller and they also have a faster rapid inactivation mechanism, consistent with the Cav1.2 channel scheme proposed by Ferreira et al. (2003) (Ferreira et al., 2003). This might be explained by an agonist binding of Pb^{2+}, promoting fast divalent inactivation of Cav1.2 channels, but reduced slow inactivation (Ferreira et al., 2003; Peterson et al., 1999; Wilson and Brunger, 2003). Although the currents through the Cav1.2 channels are smaller in the presence of Pb^{2+}, they last longer in comparison with those carried only by Ca^{2+}, similar to what has been described for inactivation by Ba^{2+}(Ferreira et al., 1997). Cav1.2 channel blockage by Pb^{2+} in the presence of mM extracellular Ca^{2+} concentrations is also reversible, and disappears when Pb^{2+} is removed from the extracellular medium (Ferreira de Mattos et al., 2017). This suggests that Pb^{2+} might also block the Cav1.2 channel on its extracellular side (Marchetti, 2013). The current-voltage curve for control and 30 μM Pb^{2+} is shown in Fig. 5C. Currents diminished at all voltages. However, currents in Pb^{2+} remained for prolonged times, a phenomenon that may be due to its different affinity for the Cav1.2 pore in comparison with Ca^{2+}. The putative pore of Cav1.2 channels is shown in Fig. 5D. Divalent cations are selected by four glutamate residues present in all the repeats of the channel at position 0 (Abderemane-Ali et al., 2019). Pb^{2+} should block the Cav1.2 channels because it has higher affinity for the glutamate residues in comparison with Ca^{2+}, and thus it should interfere with the currents carried through these channels. The blockage of Cav1.2 channels does not imply that Pb^{2+} is trapped inside these channels, but rather it implies that their passage is slower because of the different affinity between Pb^{2+} and Ca^{2+} for the Cav1.2 channel pore (Fig. 5D) (Hess et al., 1986; Lansman et al., 1986). It is also consistent with the properties of Pb^{2+} observed in these channels in other cell types (Atchison, 2003; Legare et al., 1998). For example, Pb^{2+} might have additional

targets in the cardiac Cav1.2 channels compared with Ca^{2+}(Ferreira de Mattos et al., 2017). Because Cav1.2 channels are of critical importance to heart function, any negative impact on these channels will have functional consequences on various properties of the heart (contractility, excitability and conductivity). The tension records from experiments done in similar experimental conditions to what was reported in (Ferreira de Mattos et al., 2017), are shown in Fig. 5E. The diastolic tension is enhanced by Pb^{2+}, suggesting a direct interaction of Pb^{2+} with the contractile protein complex and/or effects on intracellular Ca^{2+} homeostasis (Ferreira de Mattos et al., 2017). This in turn was associated with an slower activation and relaxation contraction kinetics (Ferreira de Mattos et al., 2017). It was hypothesized that the negative inotropic effect, in addition to the interference with the contractile machinery, could be also explained by dysregulation of Ca^{2+} homeostasis promoted by the Pb^{2+} exposome (Carmignani et al., 2000; Ferreira de Mattos et al., 2017; Prentice and Kopp, 1985; Vassallo et al., 2008). Results consistent with this are shown for measurements made in isolated guinea-pig heart cells. Contractions were measured under control conditions (Fig. 5F, *top,* black tracings) or during exposure to a range (10–30 μM) of Pb^{2+} (Fig. 5F, *top,* red tracings). Contractions were non-synchronized and highly irregular in the presence of Pb^{2+} (see the plot of varying length of the cells *versus* time). The same measurements were performed in isolated heart cells pre-loaded with a dye sensitive to intracellular Ca^{2+} and less sensitive to Pb^{2+} (Calbryte). The results of fluorescence elicited mostly by intracellular Ca^{2+} binding to the dye are shown in Fig. 5F, *bottom*. The basal/resting Ca^{2+} level is higher in cells with extracellular Pb^{2+} than in the control cells. This may explain the rise in diastolic tension. The findings suggest that Pb^{2+} plays an essential role in dysregulating Ca^{2+} homeostasis and eliciting the anomalous pattern of contractions observed in the isolated hearts and heart cells. An intriguing question is whether Ryr 2 channels can be stimulated by Pb^{2+} as they are stimulated by Ca^{2+}, and also whether the endoplasmic reticulum content of Ca^{2+} is depleted and the SERCA Ca^{2+} pump is inhibited. It seems that both mechanisms are affected by Pb^{2+}(Ferreira de Mattos et al., 2017). In addition, when the diastolic tension rises and the heart becomes more rigid in the presence of Pb^{2+}, the amplitude of contractions is also diminished (negative inotropic effect). Thus there is a reduced ability of the heart to contract, resulting in a lower amplitude of contractions because of a more rigid heart. This finding agrees with higher resting/basal levels of intracellular Ca^{2+} found when extracellular Pb^{2+} is present (Ferreira de Mattos et al., 2017).

Another final outline from experiments related to explore the direct effects by the Pb^{2+} exposome is that either by alteration of red-ox mechanisms and/or dysregulation of intracellular Ca^{2+} homeostasis, Pb^{2+} can affect various Ca^{2+}-binding proteins and biomolecules critical for heart function. For example, fast inactivation of Cav1.2 channels and several other intracellular processes in myocytes are known to be modulated by calmodulin. Pb^{2+} binds to **calmodulin** with high affinity, interfering with the processes regulated by this critical molecule in myocytes (Kirberger and Yang, 2008; Kursula and Majava, 2007; Wilson and Brunger, 2003). In addition, Na^{+} and several K^{+} channels, the NCX Na^{+}-Ca^{2+} exchanger, are important in the generation of a cardiac action potential. Channels like the ryanodine-receptor type 2 (Ryr 2), the SERCA Ca^{2+} pump, the NCX transporter, the plasma membrane Ca^{2+} pump (PMCA), and the calcium stores in mitochondria are highly relevant for maintaining the proper internal circulation of Ca^{2+} during heartbeats and controlling intracellular Ca^{2+} homeostasis. Another critical enzyme family affected by lead poisoning in the heart and many other cells are **protein-kinase-C** (PKC) (Dorn and Force, 2005; Markovac and Goldstein, 1988). Heart rate and rhythm are strongly modulated by autonomic innervation and humoral responses. The results obtained using isolated hearts from guinea-pigs, denervated and without humoral control, show that Pb^{2+} has a biphasic impact on heart rate, increasing its variability as well. In parallel, the rate of appearance of arrhythmias are also increased by acute exposure to Pb^{2+} (Ferreira de Mattos et al., 2017). The results obtained using these *ex-vivo* heart models were similar to those reported in clinical studies conducted with human populations, and they provide in addition ways of exploring in detail the molecular mechanisms involved in lead poisoning that could explain the clinical observations in humans (Alissa and Ferns, 2011; Andrzejak et al., 2004; Cheng et al., 1998).

4. Cardiac effects of the mercury exposome

All of the general cardiac effects of the Pb exposome also apply to the Hg exposome. The dose-dependent, multisystemic effects of Hg on heart function have been studied using *in-vivo*. *ex-vivo* and *in-vitro* approaches. However, the multiple forms of Hg in the Hg exposome (elemental mercury, salts, and organic mercury) and the ability of these multiple forms to interconvert between each other increase the difficulties in monitoring this heavy metal (Rani et al., 2019). Even with these difficulties, studies on the levels of Hg in human hair have been correlated with oxidation of biomolecules, such as oxidized low-density lipoproteins (LDL), that are known to be related to the presence of cardiovascular diseases (Li et al., 2008; Yoshizawa et al., 2002). Indeed, levels of Hg in hair between 1 and 2 μg/g is a good predictor of developing future cardiovascular disease (Hu et al., 2021). These and other studies have established that the Hg exposome increases the risk of cardiovascular diseases (Guallar et al., 2002; Hu et al., 2021; Mozaffarian et al., 2011; Roman et al., 2011; Virtanen et al., 2007). For example, the consumption of sea-food and fish that have accumulated Hg in areas of Hg exposome is associated with an increased risk of myocardial infarction (Grandjean et al., 2005) and cardiovascular diseases (Chan and Egeland, 2004; Guallar et al., 2002; Mozaffarian and health, 2009; Mozaffarian et al., 2011).

Hg^{2+} can produce alterations in the function of the heart by dysregulation of humoral and neuronal modulation, alterations in blood vessels, or changes in innervation. Cardiac effects *in-vivo* mediated by Hg have been reported, though these effects could also be due to the impact of Hg on neuronal and humoral systems and coronary blood vessels. For example, heart rate recovery after exercise, which is modulated by the autonomous nervous system, is impaired by Hg poisoning (Yilmaz et al., 2016).

Heart function is also known to be affected directly by the Hg exposome. *Ex-vivo* experiments using isolated guinea-pig hearts and *in-vitro* studies using cardiomyocytes isolated from guinea-pigs ventricles have been conducted with or without treatment with inorganic salts of Hg, such as $HgCl_2$, and these experiments have demonstrated show that there is a direct effect of acute Hg^{2+} exposure on the denervated heart and heart cells (Vassallo et al., 1999). In *ex-vivo* experiments the hearts lack nerve and humoral regulation and there still are damaging effects of Hg on the heart. The effects of Hg are thus independent of possible damage to coronary blood vessels. Consistently with this, the effects were also observed in single isolated cells *in-vitro* (Kamynsky et al., 2016). Hg^{2+} can enter cardiomyocytes where it interferes with red-ox mechanisms and calcium homeostasis (Fernandes Azevedo et al., 2012; Genchi et al., 2017; Mendoza et al., 2020). Experiments performed in rats suggest a biphasic effect of Hg on cardiac contraction, where there is a positive inotropic effect at very low concentrations, and a negative inotropic effect at higher doses (Oliveira et al., 1994). The effects of Hg on heart contraction are partially prevented by dithiothreitol (DTT) and cysteines (Vassallo et al., 1999), also suggesting the involvement of red-ox mechanisms.

The mechanisms by which Hg^{2+} enters into cardiac cells are unclear. *In-vitro* experiments with endothelial cells isolated from blood vessels and placed in tissue culture have recently been reported (Liu et al., 2020). The results indicate that Hg entry inside cells might involve several transporters (Valera et al., 2011) and unidentified ion channels (Liu et al., 2020). For example, certain aquaporins play a role in the entry of arsenic into cells, but they are inhibited by Hg (Savage and Stroud, 2007; Shinkai et al., 2009). Mercury transporters (ABC-type), and/or amino acid transporters have been identified and characterized

as possible pathways of entry of Hg in several cell types (Brown et al., 2002; Kiyono et al., 2009; Kolbinger et al., 2019; Usuki et al., 2017).

Isolated hearts and cardiomyocytes from guinea-pigs show prolongation of their action potentials, with similar experimental conditions to what has been reported for Pb^{2+}, using Hg^{2+} instead of Pb^{2+}. However the effect of lowering the plateau and prolonging the action potential are generally greater in the Hg^{2+}-treated cells (Fig. 6A) (unpublished results presented in scientific meetings, *in preparation*). This difference could be due to a stronger blockage by Hg^{2+} (or its conversion to Hg^{+1}) on the delayed rectifier K^+ channels (hERG and KCNQ). There are reports in agreement with this view (Leonhardt et al., 1996; Narahashi et al., 1991) (Santos Ruybal et al., 2020). Taken together these effects are consistent with observations on Pb^{2+} prolongation of the action potential and QT prolongation interval, which increase heart susceptibility to arrhythmias. With respect to Cav1.2 channels, most of the actions of Hg^{2+} can be attributed to an irreversible block of these channels, in contrast to the actions of Pb^{2+} (Fig. 6B). The current-voltage relationship for Cav1.2 channels carrying Ca^{2+} currents in the absence or presence of 20 μM Hg^{2+} is shown in Fig. 6C. Hg affects currents through Cav1.2 channels at all transmembrane voltages. Since Hg^{2+} has a large ionic radius, it is unlikely that Hg enters the cardiomyocytes through these channels and it is more likely that it just blocks them. Although Hg may not enter cardiomyocytes through Cav1.2 channels, blocking these channels will exert effects on cardiac contractility and excitability. In agreement with this, studies of contractility in isolated hearts show a negative inotropic effect of Hg (Fig. 6D), consistently with a previous report from another group (Vassallo et al., 1999). Extracellular Hg^{2+} also alters and reduces the contraction of isolated guinea-pig cardiomyocytes (Fig. 6E). Fig. 6E, *bottom,* shows the effect of extracellular Hg^{2+} in the homeostasis of intracellular Ca^{2+}. The results indicate a down-regulation of the intracellular Ca^{2+} homeostasis with similar resting Ca^{2+} levels to the control. This could be explained by less amplification of Calcium-Induced-Calcium-Release (CICR) between Cav1.2 and Ryr 2 channels in response to Hg. Hg salts promote pronounced negative chronotropic effects in animals and humans after exposure (Liu et al., 2021). However, Hg also exerts a positive chronotropic effect when applied as Hg^0 (liquid Hg), instead of various Hg salts (Risher, 1997). Either Hg or Hg salts increase heart rate variability (Gribble et al., 2015; Lim et al., 2010; Valera et al., 2011), while also exhibiting pro-arrhytmogenic effects larger than observed with similar concentrations of Pb^{2+} (Fig. 6F) (Houston, 2014; Virtanen et al., 2007, 2012). These observations might be explained by interference of Hg with HCN or Na^+ channels, which can alter chronotropism and conductivity and elicit arrhythmias.

The impact of the mercury exposome in the heart is quite relevant for public health, and the possibility of mercury toxicity has to be considered in people with cardiovascular symptoms and a likely history of exposure to Hg (Genchi et al., 2017).

5. Comparison of the lead and mercury exposomes and their cardiac effects

Some differences between the Hg and Pb exposomes arise from the unique properties of each metal, their environmental distributions, and their general molecular mechanisms of action. Lead is more widely distributed worldwide because of its intense use and presence in industrial products. Both metals can affect ecosystems through chronic or acute exposures. Since both metals are non-biodegradable, they accumulate in the environment and ecosystems that are in dynamic equilibrium between air, water, soil food, and living organisms exposed to them. There are other differences as well. Pb has a more stable oxidation state as +2 than Hg, and it can interfere and damage the cells by red-ox mechanisms but also because of its ionic similarity with Ca^{2+} that allows it to compete with Ca^{2+} in many cellular processes. Mercury promotes damage differently, especially by interfering with red-ox mechanisms that can directly alter the oxidative stress in mitochondria, and it can react with many reduced groups in proteins that are easily oxidized like –SH groups in Cysteine residues. Hg has several forms that are capable of producing damage, such as elemental mercury, salts (especially mercuric salts, +2), and organic mercury, especially MeHg. These forms of Hg have different abilities to cross the membranes and barriers in the body like the blood-brain barrier, and they can interconvert between each Hg form.

One of the main targets in animals of both the Pb and Hg exposomes is the central nervous systems of developing children and pregnant women. In addition, it should be considered that both metals have multisystemic impacts on various organisms and that a major target is the cardiovascular system. Thus, these exposomes are a risk factor for humans because of high blood pressure and cardiovascular disease. The multisystemic effects of these heavy metals on cardiac tissues can occur either by indirect (neuronal or humoral mechanisms, or effects on blood vessels) or direct mechanisms. The consequences of these exposomes on heart function by direct mechanisms, such as directly entering cardiomyocytes, has been shown in isolated hearts and cells from animal hearts.

Comparisons between the direct cardiac effects of Pb^{2+} and Hg^{2+} in isolated hearts *ex-vivo* and coronary cells *in-vitro* are shown in Tables 1A and 1B, respectively. The tables correspond partially to unpublished results in preparation from our laboratory exploring the cardiac effects of Hg^{2+}, described in this review (Section 4), following the same experimental conditions as our previous published experimental work for Pb^{2+}, (substituting extracellular Hg^{2+} for Pb^{2+}), and also experimental work analyzed from Section 3, whose experimental conditions are the same as those previously published for Pb^{2+} (Ferreira de Mattos et al., 2017). The effects reported in isolated guinea-pig hearts show that these heavy metals alter the heart excitability, contractility, heart rate and rhythm. Both heavy metals decrease cardiac excitability and contractility, though Hg^{2+} is not as potent as Pb^{2+}, as confirmed by the parameters of the related dose-response curves. However, when comparing heart rate and the ability to alter heart rhythm, Hg^{2+} is more effective than Pb^{2+}. These differences could be explained by the Ca^{2+}-like behavior of Pb^{2+} and the stronger effect of Hg^{2+} in places that bind monovalent cations. This could possibly be due to the interconversion between Hg^{2+} and Hg^{+1} forms. At the single-cell level, both heavy metals block Cav1.2 channels. In contrast to Pb^{2+}, however, Hg^{2+} reduces the contraction and its variability. Thus at larger doses, both heavy metals have a direct negative inotropic effect on the heart. Both heavy metals alter excitability and increase the heart susceptibility to arrhythmias, though their potency is not the same. The similarity in some of the observed effects can be explained, in part, by their blocking effects on Cav1.2 channels.

The first point of contact in the heart for both metals is the extracellular matrix and the cell membranes. In the heart these metals promote cellular alterations by binding to and affecting the surrounding extracellular matrix, and they also act at the level of cell membrane by modifying ion channels, transporters, and receptors. These effects have been shown in heart cell cultures and other *in-vitro* experiments. The second point of direct interaction on heart function is intracellular, and this has consequences for the contractile machinery, excitability, calcium homeostasis and heart rhythm. The effects of heavy metal damage to the heart tends to be due to both mechanisms, red-ox effects and lead interference with Ca^{2+}-mediated phenomenon, and mostly dramatic widespread red-ox effects for mercury. The blood levels of these metals are not well correlated with the observed *in-vivo* effects, especially for Pb^{2+}, because lead can be stored in bones and muscles for many years, making its overall burden difficult to determine.

The environmental effects of Pb and Hg and concerns about how these exposomes impact on public health are increasing again worldwide. The first reaction to this is to stop their extraction and ban their usage in human activities, but that precludes their importance in millions of commercial products and use for industrial production. Once identified as a contaminant, they can be removed from the environment

Table 1
Comparison between the direct cardiac effects of lead and mercury exposomes.

A)

HEAVY METAL	CARDIAC EFFECTS IN ISOLATED HEARTS			
	EXCITABILITY (APD80)	CONTRACTILITY (Tension/Tension$_{max}$)	HEART RATE (Beats/min)	ARRHYTHMIAS (Events/30 s)
Pb2+	**IC50 12 ± 1.2 μM H −1.22 ± 0.2**	**82 ± 23 μM −1.16 ± 0.38**	**16.2 ± 2 μM −6 ± 2**	**55 ± 12 μM 4 ± 0.41**
Hg2+	**IC50 17 ± 1.2 μM H −1.1 ± 0.2**	**118 ± 26 μM −1.13 ± 0.24**	**4 ± 0.5 μM −4 ± 0.2**	**48 ± 14 μM 1.12 ± 0.34**

B)

HEAVY METAL	CARDIAC EFFECTS IN CARDIOMYOCYTES		
	ICaL (Cav 1; 2)	SINGLE CELL CONTRACTION (Δlength/length)	INTRACELLULAR Ca^{2+} (ΔCa2+ dye fluorescense, ΔF/F)
$_{Pb}$2+	**IC50 38 ± 3.4 μM ^{h} -1.28 ± 0.13**	**0.18 ± 0.12 σ^2 0.56**	**25 ± 0.31 5.76**
$_{Hg}$2+	**IC50 34 ± 13 μM h -0.8 ± 0.21**	**0.14 ± 0.08 σ^2 0.45**	**20 ± 0.21 2.25**
Control N/A		**0.27 ± 0.09 σ^2 0.4**	**19.64 ± 0.34 5.95**

The comparison was made in isolated hearts and cells analyzing experments like those reported in Fig. 5 for Pb^{2+} and Fig. 6 for Hg^{2+}. The results from similar experiments with Pb^{2+} have been previously published (Ferreira de Mattos et al., 2017). The results with Hg^{2+} are unpublished though they have been presented in scientific meetings and symposia, *in preparation*. (A) The main cardiac functions were evaluated for Pb^{2+} and Hg^{2+} obtaining dose-response curves and the best fit parameters of a Hill equation to data related to excitability, contractility, heart rate and rhythm. The IC50 and h (hill number) values shown are the best parameters obtained from fitting a Hill equation to the data (R = y0 + Dy. Dˆh/(Dˆh + IC50ˆh, R = response, D = Dose, n = hill number, IC50 = half dose for maximal response, Dy = maximal response, y0 = minimal response). The monophasic Action Potential Duration at 80% repolarization (APD80) is affected by both heavy metals. Though both cations have a negative inotropic effect, this effect is more pronounced por Pb^{2+} than for Hg^{2+} (IC50 Pb^{2+} approx. 70 μM and Hg^{2+} approx. 110 μM). There is a more dramatic effect in heart rate and rhythm for Hg^{2+} than for Pb^{2}. The proarrhythmic effects are more frequent in Hg^{2+} than in Pb^{2+-}treated hearts at a particular concentration. (B) In the case of single cell excitability and contraction, we measured the best fit parameters of a Hill equation for Cav1.2 blockage and also the contractions as relative length variations (ΔL/L) and relative Ca^{2+}-binding fluorescence (ΔF/F) as the mean ($\bar{x}$) and standard error of the mean (s.e.m, $\sigma_{\bar{x}}$) obtained in a central cell line under repetitive stimulation of the same frequency in extracellular concentrations of 10–30 μM of each heavy metal or under control. Both, extracellular Pb^{2+} and Hg^{2+}, block Cav1.2 channels. The single cell contractions under pulse-field stimulation are less intense in Hg^{2+} than in Pb^{2+}-treated cells. Under pulse-field stimulation, there is a regular pattern of Ca^{2+} release in control medium. The basal intracellular Ca^{2+} and Ca^{2+} variability is increased in Pb^{2+} compared to Hg^{2+}-treated cells. (Unpublished results, *in preparation*). Summary of abbreviations and methods for this table: A. Isolated hearts were isolated from guinea pigs and disposed in a Langerdoff column as described by (Ferreira de Mattos et al., 2017). In isolated hearts, excitability was estimated from monophasic Action Potential Duration at 80% repolarization (APD80), contractility was determined as tension over time with a Weathsthone bridge tension transducer attached to the base of the papillary muscle and expressed as normalized values (T/Tmáx), heart rate was measured as beats/min and arrhythmias by the number of abnormal electrical events during an interval of time (bin). The best fit parameters for each variable in Pb^{2+} and Hg^{2+} after fitting a Hill equation (IC50 and h), are indicated below each measured variable. IC50 corresponds to the half-dose for the maximum effect and h to the hill number indicating the slope of the curve. B. Isolated cardiomyocytes were obtained from guinea-pig hearts, following modified enzymatic methods (Ferreira et al., 1997a). The variables measured were currents through the L-type Calcium current (ICaL), single cell contraction as relative variation in length (ΔL/L), and intracellular Ca^{2+} as the relative change in fluorescence intensity from a Calcium-binding dye. As in "A", each of these variables was measured with Pb^{2+} and Hg^{2+}, obtaining a dose-response curve with the parameters IC50 and h, as described before.

and ecosystems, usually in very slow processes that can take many years. Removing Pb and Hg from the environment and various ecosystems in their oxidized free states, which are the most damaging and reactive states, through different chelation procedures can be accomplished in a process known as "remediation" of the exposomes. To identify potential regions of high risk, maps of exposomes with their levels in the air, soil, water, food, and organisms (people and sentinel animals), in space and time have to be constructed, and then they have to be assessed with continuous monitoring using biomarkers or biosensors. Finally, these results have to facilitate active policies of promoting public health around the world.

6. Concluding remarks

The heavy metals Pb and Hg are a challenge for society because of their effects on the normal development of humans and animals, storage in other living forms, and their consequences on ecosystems worldwide. Also, they can accumulate persistently in the environment over many years and numerous industries still use them in different industrial processes. Exposure to Pb and Hg is usually done in small amounts and from time to time. In the body, they can be stably stored (mainly in bone). As a result the exposure even. in small amounts, should be considered. The accumulation is progressive and may reach toxic levels after years of exposure. It is quite important to state that there are no safe limits of exposure for these metals.

The epidemiological assessment of their damage presents a very complex problem, and they must be analyzed both epidemiologically and molecularly, since they have non-specific systemic impacts on people and on ecosystems. The exposome approach is a starting point to coordinate space and time observations in high-risk areas around the world. Maps with these heavy metals levels of risk of exposure as well as continuous monitoring have to be prepared worldwide.

Although most of the scientific efforts on heavy metal exposomes have been concentrated on understanding and characterizing the neurological impact of these exposomes, it has become evident that they can act either by indirect or direct mechanisms on many tissues and organs. Heavy metals should be considered as risk factors for cardiovascular diseases along with diabetes, hypertension, smoking, and other factors. Thus, individuals with cardiovascular diseases of uncertain cause must be analyzed in the context of these exposomes that can alter their cardiovascular system, increase their blood pressure, and other effects that eventually damage the heart and contribute to cardiac

diseases and eventually failure through a variety of synergistic mechanisms that ultimately could result in a fatal outcome.

Additional environmental and epidemiological research as well as basic and clinical research are needed to better understand these problems that are affecting all mankind. Some effort also has to be made to diversify the industrial production of the materials that require the use of these heavy metals in the production process and limit the exposure of workers and contamination of products and the environment.

Declarations of interest

None. The authors confirm that there is no conflict of interest.

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Declaration of competing interest

None. All the authors have declared no conflicts of interests.

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