Chapter

Nefarious, but in a Different Way: Comparing the Ecotoxicity, Gene Toxicity and Mutagenicity of Lead (Pb) and Cadmium (Cd) in the Context of Small Mammal Ecotoxicology

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Abstract

Lead and cadmium are long established toxic and carcinogenic metals. Still, the mechanisms of their interaction with eukaryotic DNA are not unequivocally understood. New data provide evidence on the influence of both metals on DNA repair, particularly non-homologous end joining (NHEJ) and mismatch repair (MMR). This may help explain the weak direct mutagenicity of both Pb^{2+} and Cd^{2+} ions in the Ames test, as opposed to the proven carcinogenicity of both metals; it has long been proposed that lead and cadmium may induce an imbalance in mammalian systems of DNA damage repair and promote genomic instability. While new evidence for mechanistic interactions of metals with DNA repair emerges, some of the old questions involving dose distribution, pathways of exposure and bioaccumulation/detoxification kinetics still remain valid. To help place the current state of the art in the genetic toxicology of lead and cadmium within the context of ecotoxicology, the current authors propose an integrative approach and offer a review of other authors' work as well as some of their own data on systemic and organ-specific toxicities in laboratory mice. The current chapter is a comparative analysis of the state of the art in the specific toxicity and genotoxicity of Pb and Cd, presenting some new and little-known information.

Keywords: lead (Pb), cadmium (Cd), genotoxicity, ecotoxicology, physiological reactions, DNA damage and repair, cell signaling, laboratory mice

1. Introduction

The last several decades have seen an increase in scientific and public interest in the problem of environmental contamination as a consequence of human activities. A wide variety of chemicals is released into the environment from different sources, either intentionally or as a result of accidents, prompting

widespread concern about the effects of anthropogenic contamination on the biota. While many organic pollutants such as pesticides and petroleum refining products are subject to environmental degradation by physical, chemical, or biological pathways, heavy metals and their compounds typically retain their toxicity over long periods of time. Recently, important advances have been made in the understanding of the gene toxicity and mutagenicity of heavy metals in the environment [1–6]. For instance, it has been established that the gene toxicity of lead (Pb^{2+}) and cadmium (Cd^{2+}) ions is not due to direct DNA-metal interactions [2, 3]. It has been demonstrated that Cd^{2+} affects DNA repair pathways, particularly the non-homologous end joining (NHEJ) of DNA double-strand breaks (DSBs) at very low concentrations (<30 μmol) in several *in vitro* test systems [4, 5]. Nevertheless, some questions regarding the gene toxicity and mutagenicity of lead and cadmium remain open. For instance, several authors have noted that *in vivo* test systems are much more sensitive than *in vitro* systems (i.e., cell cultures) with respect to lead-induced endpoints for genotoxicity assessment (chromosomal aberrations, micronuclei, sister chromatid exchanges, comet assay endpoints) [7, 8]. In practice this means that animal models, especially rodents, are much more suitable for analysis of the genotoxicity of Pb^{2+} than cell cultures. When considering cadmium, useful mechanistic data on mutagenicity and co-mutagenicity has been obtained with *in vitro* test systems [4, 5]. Still, the question of the importance of Cd^{2+} as genotoxic agent in living mammalian organisms remains open. One study has reported lead-induced genomic instability in the progeny of mice exposed to Pb^{2+} *in utero* [9]. It is still unclear if this phenomenon has been observed by other authors and how common heavy metal-induced genomic instability is. If parental exposure to toxic metals can influence the stability of the genome in subsequent generations, this is potentially very alarming and could influence the current standards and permissible limits for occupational and environmental exposure. Last but not least, toxic metals seldom occur alone in contaminated sites. For instance, non-ferrous metal smelters typically emit a cocktail of toxic chemical elements in the atmosphere. This means that an accurate environmental risk assessment should be performed on a case-by-case basis, and that both ecotoxicological biomonitoring, and more general attempts to resolve the problem of heavy metal genotoxicity and mutagenicity, should be concerned not with a single toxicant but rather a plurality of different toxic agents present in a given locality. A number of studies have been performed with wild rodents exposed environmentally to complex contamination including Pb^{2+} and Cd^{2+} [10–23]. While these studies include endpoints for scoring genetic damage (chromosomal aberrations, micronuclei, comet tail length and tail moment) relatively little is understood about the molecular mechanisms underpinning the genotoxicity of complex mixtures of toxic metals.

In summary, from the perspective of ecotoxicology, it is well-established that Cd^{2+} and Pb^{2+} are genotoxic metal ions, especially in complex organisms. At the same time, knowledge about the mechanisms for heavy metal genotoxicity is scarce, with anecdotal evidence for interactions with DNA repair systems in complex vertebrate organisms, and relatively little knowledge of how the gene toxicity of Pb^{2+} and Cd^{2+} fits into the bigger picture of the specific physiological reactions of terrestrial vertebrates to toxic metals. For the purposes of the current study, the main questions regarding lead and cadmium gene toxicity are the following:

1. What are the specific molecular mechanisms, responsible for the gene toxicity of Pb^{2+} and Cd^{2+}? How does intoxication with heavy metals lead to detectable chromosomal damage and mutagenesis? What are the similarities and differences when considering the gene toxicity of lead and cadmium?

2. Can we draw conclusions about the comprehensive biological effects of heavy metals? For instance, it has long been established that terrestrial vertebrates respond to Pb^{2+} and Cd^{2+} by increased expression of detoxifying proteins (metallothioneins) and increased biosynthesis of glutathione. While there is evidence for adaptive responses, how does this apply to genetic damage induced by heavy metals?

3. What are the effects of complex environmental pollution? How do complex mixtures of metallic toxicants affect organisms?

4. Last but not least, what are the prospects, challenges, and potential answers from future studies dealing with the gene toxicity of Pb^{2+} and Cd^{2+}?

In order to provide, in part, answers to these four questions, the current study aims to analyze the state-of-the-art in what is known about the genotoxicity of lead and cadmium within the context of ecotoxicology. The current authors have employed a wide scope of sources in order to synthesize what is currently known and understood about the gene toxicity of Pb^{2+} and Cd^{2+}, and conduct a comparative analysis of the two metals. In addition, insight and information is provided from a personal set of sources and experience, which are not widely publicized. Finally, the current article discusses several potential directions for future studies in the gene toxicity of heavy metals and proposes an integrated, trans-disciplinary approach to solving the problems, associated with the ecotoxicity and gene toxicity of Pb^{2+} and Cd^{2+}.

2. Lead (Pb)

2.1 Ecotoxicity, bioaccumulation patterns, and specific organ toxicities

Lead (Pb) is present in the Earth's crust at comparatively low concentrations (0.121 ppb) and has four stable isotopes (^{204}Pb, ^{206}Pb, ^{107}Pb, and ^{208}Pb) [24]. Although a comparatively rare metal, it has been historically one of the first industrially mined chemical elements. Contemporary sources estimate annual primary production of lead to be 4.6 million metric tons [25]. While Pb has been released in the atmosphere during manufacturing processes and combustion of fossil fuels, leading to global trace contamination, the main concern has been strong local contamination in the vicinity of mining, refining and smelting processes, as well as localized accidental releases. The toxicity of lead has been suspected since ancient times, with authors arguing mass poisoning from the metal in Ancient Rome due to its use for water pipes, glassmaking, and in winemaking processes [26]. Contemporary ecotoxicological research is concerned mainly with local contamination with Pb, with several important impact sites identified in Europe: Bukowno in Poland, Nitra, Slovakia, Asenovgrad, Bulgaria, and the Coto Doñana area in Spain [12, 15, 16, 18, 19, 22, 27–29]. The studies in these areas have dealt mainly with biomonitor species of wild rodents, and have investigated bioaccumulation of lead and other toxic metals, as well as endpoints for the determination of gene toxicity. Regardless of the zoomonitor used (typically, the wood mouse, *Apodemus sylvaticus*, yellow-necked mouse, *Apodemus flavicollis*, bank vole, *Myodes glareolus*, common vole, *Microtus arvalis*, Algerian mouse, *Mus spretus*), similar tendencies for bioaccumulation of Pb in the organisms of small mammals have been detected, and often correlated with the induction of genetic damage (chromosome aberrations, micronuclei). These studies have demonstrated significant effects of heavy metal

contamination on the biota, and have proven the importance of continuing monitoring studies in contaminated ecosystems.

The biokinetics and specific organ and tissue toxicities of Pb have been actively investigated in animal models since the late 1950s, initially employing radioactive tracer isotopes such as ^{203}Pb and ^{210}Pb [30, 31]. This has led to the development of several biokinetic models for the metal in mammalian organisms [31–33]. The Harley-Kneip six-compartent model, developed with the use of primates, is considered to be one of the first informative biokinetic models for lead absorption, distribution and elimination (**Figure 1**).

As evident from the model, a significant percentage of ingested lead (~80%) is excreted without being absorbed by the gut. At the same time, the coefficient for absorption from the bloodstream into bone λ_{12} = 0.34–0.11 is significantly higher than the coefficient for release of Pb from the bones into the bloodstream (λ_{21} = 1.73 x 10^{-3}). In practice, this means that once a significant amount of lead is absorbed into the bones, it is practically impossible to eliminate it. The Harley-Kneip model also emphasizes the differences between juvenile and adult organisms, with juvenile animals much more susceptible to lead bioaccumulation [32]. To a varying level, Pb is also absorbed in the liver, kidneys, and the nervous system. It has been established that, in mammalian organisms, if the metal reaches sustained blood levels above 80 μg/dL, practically every organ and system is affected [24].

The primary targets for lead intoxication are the hematopoietic system, the nervous system and the liver. At sustained blood levels above 50 μg/dL, Pb inhibits

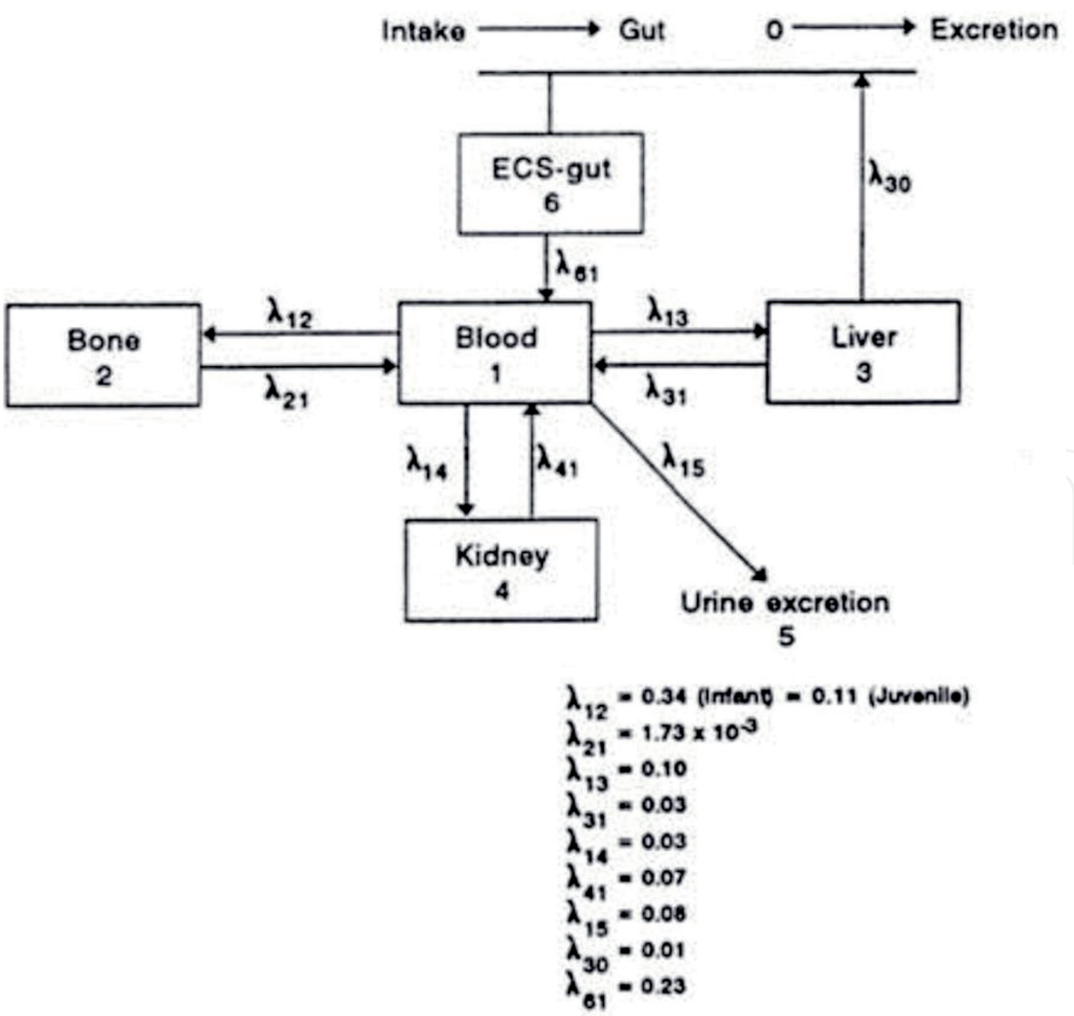

Figure 1.
Biokinetic model for the metabolism of lead in mammalian organisms [32].

the enzymes delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase, leading to impaired erythrocyte biogenesis, disturbances in erythrocyte kinetics, and anemia [34]. Several authors report an inhibition of the immune system at blood levels above 50 μg/dL, as well as histopathological lesions in the bone marrow at levels exceeding 100 μg/dL [23, 35, 36]. Death from anemia occurs at blood levels above 150 μg/dL. The nervous system is particularly sensitive in young individuals, and it has been established that Pb levels in blood exceeding 18 μg/dL lead to cognitive disturbances; it has been reported that very low doses cause neuronal apoptosis in rats [37]. In cases of chronic and sub-chronic lead intoxication, there is significant liver damage. Macroscopically, the liver increases in size; steatosis, hyperplasia and disruption of the liver microvasculature, as well as focal necrosis, have been observed at doses above 40 μg/dL, with marked changes in the activity of alanine and aspartate transaminase (ALT, and AST) and kidney damage [8].

2.2 Gene toxicity and mutagenicity

Due to low direct mutagenicity levels in the Ames test, lead (Pb) was initially thought not to be directly mutagenic [38]. Nevertheless, evidence soon accumulated that the metal was responsible for producing chromosomal aberrations in occupationally exposed workers and environmentally exposed human populations [31, 35]. Since the 1970s different *in vitro* and *in vivo* studies have been conducted regarding the potential of lead compounds to damage genomic DNA in mammals. The table below presents several informative studies conducted on the gene toxicity of lead using different *in vitro* test systems and endpoints, arranged chronologically (**Table 1**).

The studies cited provide evidence that lead is mutagenic and clastogenic under certain circumstances. While older studies show relatively weak clastogenicity of Pb when considering chromosomal aberrations [39, 40], newer publications report genotoxicity by using more sensitive endpoints, such as the induction of sister chromatid exchanges (SCE), tail length in the comet assay, and induction of γH2AX foci, indicating DNA double-strand breaks [6, 43, 44]. It should be noted that the study indicating the highest toxicity of Pb, uses lead chromate ($PbCrO_4$), which means its effects could be due to the inherent gene toxicity of hexavalent chromium [44].

Several authors have noted the greater sensitivity of *in vivo* test systems when studying the gene toxicity of lead [7, 9]. For the purposes of the current study, several sources dealing with *in vivo* models have been selected (**Table 2**).

It should be noted that, in contrast to *in vitro* test systems not almost all tests with Pb administration to living animals show evidence for genotoxic effects. Not only that, some authors have noted a very close dose dependence of effects on Pb concentrations in living organisms, as well as trans-generational accumulation of chromosomal aberrations after exposure of mice *in utero* [9]. From the viewpoint of ecotoxicology, this means that the risks from environmental exposure to lead compounds are often underestimated when using *in vitro* test systems and only *in vivo* models can provide an accurate assessment of genetic risk to the biota.

Much discussion has taken place concerning the molecular mechanisms of Pb-induced genetic damage. For instance, in the last two decades it has been accepted that lead interferes with the mechanisms for DNA repair, which is evident with studies analyzing Pb as a co-mutagen with other agents such as UV light, X-rays and methylnitronitrosoguanidine (MNNG) [50]. While it is accepter that the metal can inhibit DNA repair, the mechanisms of DNA damage induction *per se* are not well understood. For instance, it has been conclusively demonstrated that Pb and Cd do not interact with DNA directly under physiological conditions [3]. On the other hand, other authors have noted that Pb and other toxic metals can

Authors	Substance tested	Test system	Dose	Exposure	Endpoint	Effect
Bauchinger and Schmid [39]	Lead acetate	CHO cells	10^{-6} to 10^{-3} M	16 h	CA	No effect, except for increase of gaps
Gasiorek and Bauchinger [40]	Lead acetate	lymphocytes	10^{-3} to 10^{-5} M	3 h	CA	No effect
Hartwig et al. [41]	Lead acetate	CH V79 cells	0.5–10 μM	44 h	HPRT mutation	Co-mutagenicity with UV light
					SCE	Increase in SCE
Cai and Arenaz [42]	Lead nitrate	CHO AA8 cells	10^{-6} to 10^{-8} M	48–60 h	CA	No effect
					SCE	Increase in SCE
Wozniak and Blasiak [43]	Lead acetate	lymphocytes	1–100 μM	1 h	Comet assay	Increase in tail length and % tail DNA
Xie et al. [44]	Lead chromate	lung fibroblasts	0.1–5 μM	24–48 h	CA	Increase of % metaphases with damage
					Comet assay	Increased % tail DNA
					γH2AX foci	Dose-dependent increase of γH2AX foci
Pottier et al. [6]	Lead nitrate	EJ30 carcinoma	30–1000 μM	24 h	γH2AX foci	Dose-dependent increase of γH2AX foci
					Telomere score	Telomere instability

Table 1.
Exemplary studies on the genotoxicity of lead compounds in vitro.

induce a pro-oxidative state in living organisms at comparatively low concentrations (<30–50 μmol) [50, 51]. In summary, it can be said that the genotoxicity of lead works at the following levels:

1. Induction of reactive oxygen species (ROS) by Fenton-like reactions; inhibition of key enzymes like glutathione-S-transferase (GST); disruption of lysosomal membranes and induction of apoptosis [51].

2. Induction of genomic DNA damage; inhibition of key DNA repair systems such as base excision repair (BER) and disruption of telomere maintenance [6].

3. Mutagenesis, clastogenesis, tumor initiation and promotion, increase in the levels of apoptosis in some tissues, reproductive toxicity, organ and system toxicities [37].

While the basics of lead genotoxicity have been confirmed, and the metal has been confirmed as reproductively toxic and carcinogenic in mammalian species,

Authors	Substance tested	Test system	Dose	Exposure	Endpoint	Effect
Muro and Goyer [45]	Lead acetate	a/SW mice	1% Pb in food	2 weeks	CA	Increase in CA
Deknudt et al. [46]	Lead acetate	*Macaca fascicularis*	1–15 mg/kg Pb in food	3–16 months	CA	Increase in CA
Sharma et al. [47]	Lead acetate	ICR mice	50–200 mg/kg PB intraperitoneally	Injection	SCE	Increase in SCE
Robbiano et al. [48]	Lead acetate	Sprague-Dawley rats	117 mg/kg in food	3 days	MN test	Increase in MN frequency
Valverde et al. [49]	Lead acetate	CD-1 mice	0.01–1 μM	Inhalation, 3 days	Comet assay	Increase in tail length
Yuan and Tang [9]	Lead acetate	Kunming mice	1 mg/l in drinking water	90 days	Comet assay	Increase in tail length
Tapisso et al. [21]	Lead acetate	*Mus spretus*	21.5 mg/kg Pb in food	17 days	MN test	Increase in MN frequency
					SCE	Increase in SCE

Table 2.
Exemplary studies on the genotoxicity of lead compounds in vivo.

much remains to be investigated regarding the molecular mechanisms of the interactions of Pb^{2+} with mammalian DNA repair systems.

3. Cadmium (Cd)

3.1 Ecotoxicity, bioaccumulation patterns, and specific organ toxicities

Cadmium (Cd) is a malleable, silvery-white metal present in the Earth's crust in concentrations of 01–0.5 ppm, having five stable isotopes (^{108}Cd, ^{110}Cd, ^{111}Cd, ^{112}Cd, and ^{114}Cd) [24]. Discovered as a separate element within zinc ores in 1817, it is a toxicant, associated primarily with the late industrial age. Mined at a large scale since the 1920s, the metal is currently produced at a level of 23,000–24,000 metric tons per year [25]. Similarly to lead, the main concern regarding Cd-associated contamination is local pollution of terrestrial and riverine ecosystems. The toxicity of cadmium was discovered after the start of its extraction from polymetallic ores, with one example being the "itai-itai" disease in the Toyama prefecture of Japan, attributed after 1950 to Cd poisoning [52]. In Europe sites, severely polluted with cadmium are comparatively rare. One exception is the area of Bukowno in Poland, where there is significant local contamination [16, 53, 54]. Several studies deal with the ecotoxicity of Cd with the use of zoomonitors (mainly yellow-necked mice, *Ap. flavicollis* and bank voles, *M. glareolus,* but also the common magpie, *Pica pica*) [53, 54]. While in Europe the element is mostly present as a trace contaminant in cases of polymetallic pollution, the main concern for cadmium contamination are the countries where most of it is mined and produced, namely China, South Korea, Japan, Mexico, Canada and Kazakhstan.

The toxicity of cadmium was discovered after animal studies in the period 1955–1970 [52, 55, 56]. In mammalian organisms, the metal affects primarily the kidneys, liver, pancreas, and, at higher levels, the nervous system [55]. As an established IARC Group 1 carcinogen, Cd increases the risk of lung cancer at low doses, and causes pneumonitis and lung edema at higher doses [52]. Nevertheless, the main target organ for chronic Cd intoxication are the kidneys, where the metal is accumulated, causing proteinuria, hypophosphatemia, histopathological changes in the kidney tissue, and loss of kidney function [57]. High chronic and sub-chronic dose burdens cause histopathological changes in the liver [58, 59]. Due to its antagonistic and antimetabolic activity against necessary elements such as Zn, Cu, and Ca, as well as its interference with a variety of DNA-binding enzymes, cadmium is considered toxic at high levels to all organs and systems [24, 57]. Unlike Pb, which has a strong tendency for bioaccumulation in the animal organism, Cd has higher rates of clearance from mammalian organisms due to the action of metallothionein (MT) proteins—low molecular-weight, highly conserved molecules, which bind non-specifically to dietary elements such as Zn, Se, Cu, as well as toxic elements like Cd, Hg, Ag, As, and, to a much lesser extent, Pb [54, 60]. Metallothioneins bind Cd^{2+} ions in mammals, form Cd-MT complexes, which are excreted through the kidneys, thereby detoxifying, to some extent, low levels of cadmium. Nevertheless, although this system is inducible and upregulated by the presence of toxic metals in the body, it gets saturated at high doses, being unable to compensate high dose burdens of toxic metals [54]. Due to the inefficiency of existing biological detoxication systems, as well as the tendency of the metal for bioaccumulation in plants and animals, Cd is considered very dangerous even at low doses where no physical symptoms are present. It is, therefore, not surprising that a variety of biomonitoring studies for Cd have been conducted [22, 61].

3.2 Gene toxicity and mutagenicity

The debate regarding the genotoxicity of cadmium continued for decades until recently [52]. This was due primarily to the fact that initially, using the Ames test, Cd was demonstrated to have very low mutagenicity. This, on the other hand, contradicted data demonstrating that the metal was a powerful carcinogen in mammals [24, 62]. At the same time, cadmium-induced inhibition of DNA repair systems and, consequently, co-genotoxicity, has been reported consistently since the late 1980s [56, 63]. Due to these relatively early observations on DNA repair inhibition, most *in vitro* studies have focused on the role of Cd as a co-genotoxin when combined with other genotoxic agents, for instance, ionizing and UV radiation, DNA intercalators and DNA alkylating agents [5, 63]. Data on cadmium-induced genotoxicity from several investigations with *in vitro* test models are presented in **Table 3**.

All the studies cited typically provide evidence for co-mutagenicity of Cd with known mutagens such as UV light, DNA alkylating agents such as methylnitronitrosoguanidine (MNNG), and ionizing radiation. Comparably to *in vitro* studies with Pb, older experimental work with cadmium provides evidence for co-mutagenicity (although not direct mutagenicity) of the metal, while newer work, utilizing more sensitive endpoints, provides evidence for specific mechanisms such as DNA repair inhibition [4, 5].

While *in vitro* studies highlight Cd as a powerful co-mutagen due to DNA repair inhibition, several *in vivo* studies have shown that cadmium can be genotoxic (particularly clastogenic) at low doses. The results of several such investigations are presented in **Table 4**.

The *in vivo* studies above demonstrate cadmium genotoxicity at acute sublethal doses. It should be noted that in these studies, no separate co-mutagen is required,

unlike in the *in vitro* models. Even though they prove conclusively that cadmium is genotoxic to mammals, they have a major shortcoming from an ecotoxicological point of view. Namely, the dose administration is either by injection or by oral gavage, which means that the observed effects of cadmium are due to acute exposure, as opposed to chronic and sub-chronic intoxication, which can be achieved by dosing the animal with food, water, or by inhalation means. One of the studies deals with minisatellite DNA instability, demonstrating that Cd intoxication can lead to instability in the non-coding segments of mammalian genomic DNA [69]. Nevertheless, this methodology is still very controversial.

To some extent, the molecular mechanisms of DNA damage induction by Cd^{2+} ions are better understood than those of Pb^{2+}-induced gene toxicity. It has been demonstrated that, at doses above 30 μM, cadmium down-regulates a key system for DNA DSB repair, namely non-homologous end-joining [4, 5]. Evidence suggests that the kinetics and formation of γH2AX foci are impaired at doses greater than 30 μM, with DNA-PKcs catalytic activity falling off at cadmium concentrations at doses of 200 μM [4, 5]. It has been established, as well, that at these doses the metal initially over-activates the system of homologous recombination repair, which may promote genomic instability [4]. Nevertheless, the induction of DNA damage in *in vivo* models by cadmium alone does not show a clear dose-response curve [52]. El-Ghor et al. have demonstrated a significant increase in microsatellite instability in rats exposed to cadmium [69]. Nevertheless, this methodology is controversial, both due to the unknown relationship of microsatellite DNA stability to the overall

Authors	Substance tested	Test system	Dose	Exposure	Endpoint	Effect
Takahashi et al. [64]	Cadmium chloride	*E. coli* CHS26	10^{-8} to 10^{-4} M	4 h	Mutagenicity	β-Gal gene inactivation
Nocentini [56]	Cadmium chloride	Human fibroblasts	10^{-7} to 10^{-2} M	24 h	DNA repair	Inhibition of DNA DSB repair
					DNA synthesis	Inhibition of DNA synthesis
Snyder et al. [65]	Cadmium chloride	HeLa cells	10^{-8} to 10^{-3} M	24 h	UV damage	Co-mutagenicity with UV light
					X-ray damage	Inhibition of DNA DSB repair
Viau et al. [4]	Cadmium chloride, cadmium acetate	HMEC-1 endothelial cells	1–100 μM	24 h	NHEJ activity	Inhibition of DNA DSB repair by NHEJ
					HR activity	Upregulation of homologous recombination
Pereira et al. [5]	Cadmium chloride	ZF-4 zebrafish cells	1–100 μM	24 h	γH2AX foci	Disruption of γH2AX foci kinetics
					Micronuclei	Dose-dependent increase of micronuclei
					DNA repair	Inhibition at doses above 30 μM

Table 3.
Exemplary studies on the genotoxicity of cadmium compounds in vitro.

Authors	Substance tested	Test system	Dose	Exposure	Endpoint	Effect
Mukherjee et al. [66]	Cadmium chloride	Swiss albino mice	0.4–6.75 mg/kg body weight	Injection	SCE	Increase in SCE
					CA	Increase in CA
					MN test	Increase in MN frequency
Privezentsev et al. [67]	Cadmium chloride	ICR mice	1 mg/kg body weight	Injection	MN test	Increase in MN frequency
					CA	Increase in CA
Fahmy and Aly [68]	Cadmium chloride	Swiss albino mice	1–7.6 mg/kg body weight	Injection	SCE	Increase in SCE
					CA	Increase in CA
El-Ghor et al. [69]	Cadmium chloride	Wistar rats	2.93 mg/kg body weight	Oral gavage	Minisatellite DNA	Minisatellite instability
Wada et al. [70]	Cadmium chloride	Sprague-Dawley rats	40–80 mg/kg body weight	Oral gavage	Comet assay	Increase in tail length

Table 4.
Exemplary studies on the genotoxicity of cadmium compounds in vivo.

stability of coding genomic DNA, and the method of Cd intoxication used (oral gavage versus the more common method of administering via food or water). The available literature leads the current authors to believe that cadmium acts as a *tumor promoter*, with initiating events being diverse other factors (ionizing radiation background, metabolic reactive oxygen species, or other genotoxic factors). With respect to reproductive toxicity and cadmium-induced genomic instability, there is reason to believe that cadmium is reproductively toxic at high doses and can cause transmissible genetic damage in the progeny of exposed individuals. Still, much more research (both mechanistic studies and eco-toxicological experimentation) is needed to demonstrate conclusively the potential of the metal to change the genetic structure of exposed populations.

4. Comparing lead and cadmium as genotoxic agents

4.1 Induction of DNA damage

It has been demonstrated that both Pb and Cd do not bind DNA directly, nor induce DNA damage due to DNA-metal interactions [3, 41]. At the same time, it is well-established that the metals promote the generation of reactive oxygen species and interact with redox signaling, disrupting cell homeostasis in organs and tissues

and promoting a pro-oxidative state [41, 71]. In addition, specific target enzymes for Cd^{2+} have been identified—these include specifically several zinc-finger proteins like p53, XPA, PARP-1 and NF-κB. This would indicate increased potential of cadmium ions to act as tumor promoters even at low concentrations [41, 71].

On the other hand, it has been observed that Cd alone, at physiological concentrations, is a more significant causal agent of chromosomal aberrations in *in vivo* models, thereby acting more strongly as a mutagen and clastogen [3]. This is probably due to stronger induction of ROS and disruption of cellular redox signaling [72].

4.2 Interactions with DNA repair systems

Little is understood about the interactions of lead with DNA repair systems. While several studies show disruption of γH2AX foci kinetics and, therefore, disruption of DNA DSB repair, and one study highlights a disruption of telomere maintenance, no mechanistic data exists to suggest how exactly Pb^{2+} ions interfere with DNA repair and the DNA damage response [6, 44].

Much more is known about the influence of Cd^{2+} ions with DNA repair. For instance, the tendency of this metal ion to displace zinc from zinc-finger DNA-binding enzymes leads to a disruption in the nucleotide-excision repair system (NER), which can explain the co-mutagenicity of cadmium with agents such as UV light and DNA alkylating chemicals [56, 63]. There have been a few studies analyzing the effects of cadmium on key DNA DSB repair systems [4, 5]. What these authors have established that, in selected *in vitro* models, even at concentrations lower than 30 μM, cadmium chloride inhibits non-homologous end-joining (NHEJ), over-activates the MRE-11-dependent homologous recombination (HR) and telomere maintenance, and leads to a general disturbance in γH2AX foci kinetics (a very sensitive indicator for DNA damage and repair), as well as a sharp decrease in DNA-PKcs catalytic activity, indicating inability to repair double-strand breaks.

While cadmium has undoubtedly been better studied as a genotoxic and co-genotoxic agent, lead (Pb) is also a significant genotoxin, albeit at significantly higher concentrations (>10-fold or more). Pointing out the exact mechanisms of the interaction of Pb with mammalian DNA repair system remains a valid topical area for future research.

5. Gene toxicity of lead and cadmium in the context of ecotoxicology

Mechanistic studies, both *in vivo* and *in vitro*, are informative when trying to understand the basic principles of heavy metal genotoxicity. Nevertheless, what is the significance of environmental exposure to Pb and Cd? Typically environmental exposure occurs chronically or sub-chronically through food, drinking water and inhalation, and happens at comparatively low doses. In addition, exposure patterns are complex. For instance, pollution is often polymetallic, with an added variety of other organic and inorganic chemicals. Studies have been conducted in localities where pollution from lead-zinc smelters and mines is present, such as Asenovgrad in Bulgaria and Bukowno in Poland [10, 12, 14, 16, 18, 22, 27] as well as in areas, polluted by ecological accidents [15, 19].

The answers that these studies give us is that each studied locality has its own pollution pattern, leading to its own "fingerprint" of systemic toxicity and gene toxicity. For instance, it has been demonstrated that for BALB/c laboratory mice, exposed to 1% polymetallic industrial dust through food, the contents of the heavy

metals Pb and Cd increase steadily in a 90-day experiment, while at the same time the incidence of chromosome aberrations peaks at the 45-day midpoint, indicating the possibility of an adaptive response [18]. Similar results have been obtained with wild rodents from the same locality in different time frames [20]. Another area of research, which is currently active and productive, is heavy metal detoxification, particularly with the use of zeolite sorbents [29]. From the viewpoint of ecotoxicology, it is already known how chronic and sub-chronic doses of Pb and Cd affect the organism separately, but more research (including mechanistic studies) is needed in order to understand the effects of complex pollution patterns on living organisms.

The available data on the gene toxicity and eco-toxicity of Pb and Cd leads the current authors to believe that more significant research needs to be done in two main areas:

1. Mechanistic studies dealing with the specific effects of the two metals on DNA repair systems. This is especially true for Pb, since lead-induced chromosomal aberrations in mammalian cells at low doses are a well-established fact, but no concrete mechanistic studies on the effects of Pb on DNA repair systems have been conducted.

2. Ecotoxicological studies highlighting the effects of different cocktails of pollutants in a given locality on a standardized test system. Suitable *in vitro* systems, which have been proposed include metabolically competent human and rat hepatoma cell lines, which have been used for the study of metabolically activated genotoxins for over two decades [73].

Finally, connections should be made to existing occupational safety and environmental legislation regarding the use of Pb and Cd worldwide. Some of the safety concerns regarding the two elements stem from the fact that heavy metals and their compounds are highly persistent in the environment. Additionally, gene toxicity, especially in the case of cadmium, have caused EU authorities to propose banning the use, mining and refining of Cd within the EU entirely. Since effects of Pb and Cd on genomic instability in the progeny of mammalian species have been observed [9, 69], but are not well understood, it is advisable that safety approaches to Cd and Pb have a "conservative approach," meaning that exposure tolerance limits and environmental releases should be as low as possible in order to mitigate risk to humans and the biosphere.

6. Conclusion

The current work has analyzed the state-of-the art in what is known about the gene toxicity of lead and cadmium in an ecotoxicological context. Cd has been demonstrated as a powerful co-mutagen in *in vitro* test systems and as a direct mutagen *in vivo*. While Pb is generally a less potent inductor of chromosome aberrations, it has still been demonstrated to be genotoxic, particularly *in vivo*. While many studies have been conducted on the environmental exposure to Pb and Cd and their compounds, the interactions of the two metals as genotoxic agents are not yet fully understood. Two main challenges remain for future research in ecotoxicology and toxicogenetics: the combination of mechanistic *in vivo* and *in vitro* studies with ecotoxicological research, in order to understand better the specific pathways of heavy metal-induced gene toxicity, and future research on the detoxication of Pb and Cd and the mitigation of their gene toxicity.

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