



# Lizards as model organisms to evaluate environmental contamination and biomonitoring

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**Abstract** Environmental contamination is reaching ever higher levels and affecting several animal populations, including humans. In this context, studies are being developed to monitor and evaluate this environmental problem using bioindicators organisms, in addition to testing the toxicity of contaminants in the laboratory. In this perspective, reptiles are ideal animals for these types of studies, considering that they are ectothermic and have a slower metabolism directly influencing their recovery power, and therefore, they are more sensitive to xenobiotic effects. Among reptiles, lizards are animals that adapt to various environmental conditions, even being found in areas with arid characteristics. Therefore, a literature review was conducted in this study regarding the use of lizards as models for ecotoxicological studies, including biomonitoring, carried out in the last 10 years, with the aim of evaluating them as bioindicators in Brazilian semi-arid region. Studies were found involving ten lizard families, among which the

most investigated was Lacertidae. The studies were classified into two categories: organic contaminants (pesticides, petroleum by-products, and explosives) and inorganic contaminants (metals such as zinc, lead and aluminum, and radionuclides). Contaminants directly contributed to DNA damage and to increasing the frequency of micronuclei in exposed animals, histopathological effects, and oxidative stress. The performed analysis highlights the usefulness of lizards as environmental biomonitors. However, the response profile is dependent on the exposure level and route, in addition to the environmental scenario analyzed. Therefore, future studies aimed at evaluating environmental contaminants are required under exposure conditions more related to the environmental reality to be studied.

**Keywords** Bioindicators · Environmental monitoring · Organic contaminants · Pesticides · Inorganic contaminants

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## Introduction

Environmental contamination at a global level has reached worrying levels, and therefore, studies have been developed in order to monitor and evaluate this problem (Poletta et al. 2008; Márquez-Ferrando et al. 2009; Marcon et al. 2010; Al-Hashem 2011; Cabarcas-Montalvo et al. 2012; Zocche et al. 2013; Schaumburg et al. 2016; Nasri et al. 2017). A wide variety of terrestrial and aquatic organisms have been used as tools to assess the environmental impacts linked to pollution in

the different environmental compartments to understand the effects of chemical agents on biota (Zapata et al. 2016). Among these organisms, reptiles and especially lizards stand out, since environmental pollution together with global climate changes and the unsustainable use of natural resources constitute the most significant threats to the populations of these animal species (Gibbons et al. 2000).

Organisms that are reactive to environmental contamination, with the exception of humans, can be considered apt to be evaluation models, as long as they are found in abundance in the area, have a low migration rate and a reduced displacement area, with this being the case for most lizards (Zocche et al. 2013). In addition, these animals are ectothermic and present a slower metabolism; therefore, they are more sensitive to toxic and xenobiotic agents, which directly influence their recovery power (Schaumburg et al. 2012). Animals in general are exposed to various types of pollution daily, many of them of anthropic origin, but there are also those of natural origin, and they constantly need to adapt to survive in the environment (Hopkins 2000). Reptiles are specifically sensitive to environmental pollutants, being exposed by several routes from the possibility of ingesting part of contaminated soil, water, or food, as well as dermal contact on contaminated substrates and gas inhalation (Burger et al. 2004; Márquez-Ferrando et al. 2009; Simonyan et al. 2018). The ingestion of contaminated soil particles is a situation that has been causing serious risks to animals, since studies have already shown that lizards living in radioactive areas or contaminated with hydrocarbons presented damage to their DNA by ingesting soil particles during feeding, a situation which can cause further damage later (Marsili et al. 2009; Al-Hashem 2011). Contaminated foods are one of the most relevant sources of exposure to toxic agents, considering that there may be bioaccumulation of chemical elements of metallic origin or even organic contaminants such as agrochemicals via the food chain, thereby generating an accumulation cycle within the organisms (Nasri et al. 2017). Finally, dermal contamination is an important entry point for pesticides and airborne pollutants, as well as contaminants in the substrate itself (Zocche et al. 2013). Therefore, all of these factors can be considered as means of monitoring and evaluating various chemical substances found in the environment.

The investigation level of ecotoxicological studies using lizards has been deepened to better assess and demonstrate the consequences of environmental contamination (Campbell and Campbell 2002). These authors introduced a synthesis showing the scarcity of studies, which used reptiles of the Squamata group to assess environmental contamination. Based on this principle, reptiles have been considered good biomonitors to assess environmental contamination given that they have reduced mobility, they do not normally live in large areas, and it is possible to assess the effective exposure in a given area (Burger et al. 2004); however, it is argued that there is a lack of studies with these animals when it comes to biomonitoring studies, mainly in relation to the effects generated by environmental contaminants in this group (Mitchelmore et al. 2005; Zapata et al. 2016; Sargsyan et al. 2018). This is because lizards have great potential as bioindicator organisms to assess the environmental risk of several areas, as well as being shown to be excellent examples with their application in ecotoxicological studies (Simonyan et al. 2018). Lizards are usually associated with a specific habitat, thus being more vulnerable to environmental degradation than other vertebrates such as birds and mammals (Hopkins 2000). Therefore, this review article aims to qualitatively and quantitatively assess current knowledge worldwide about the relevance of using lizards as biomonitors and model organisms in environmental contamination studies.

## Material and methods

Articles were searched about environmental biomonitoring and experimental models using lizards for analyzing environmental contaminants carried out in the period 2009 to 2019, thus contemplating the studies published on environmental contaminants in lizards in the last 10 years. It is worth mentioning that studies, which did not indicate that they are inserted in this required group and which were not related to any specific contaminant, were not included. The searches were carried out in the following databases: PubMed, Web of Science, Scielo, and Google Scholar in the mentioned period, using the following combinations of keywords: biomonitoring + lizards + contaminants, biomonitoring + lizards + heavy metals, lizards + contamination, lizards + pollution, lizards + pesticides, and lizards + persistent organic contaminants + pollution. The

obtained results were divided into two categories according to their origin: organic contaminants and inorganic contaminants. The performed review shows where we are in relation to ecotoxicological research using lizard species, presenting a view of the deficiencies and gaps which still need to be filled.

**Results and discussion**

A total of 30 studies using lizards as biomonitors and/or model organisms for assessing environmental contaminants have been found for the past 10 years (2009–2019), which represent samples from five continents (Tabela 1). The lizard family most studied in ecotoxicology from the total of these works was the Lacertidae family, represented in 57% of the studies. This lizard family is part of the Scincomorpha infraorder and is distributed in Africa, Asia, and Europe (Uetz and Hošek 2016). The species most frequently found in the studies was the *Podarcis sicula* lacertid with 20% of the detected records. It is a species native to Italy and widespread throughout the country, being considered quite abundant; however, because it was introduced in

other areas it can be found in other countries such as Portugal, Spain, France, Montenegro, Croatia, Turkey, Libya, and Tunisia, all being geographically close to Italy (Verderame and Scudiero 2019a).

The results obtained through this survey were divided into two categories: contaminants of organic origin (pesticides, petroleum products, coal, and explosives) and contaminants of inorganic origin (heavy metals and radionuclides; Table 1).

Organic contaminants

Lizards are an important target group of vertebrate animals exposed to various pesticides (fungicides, insecticides, and herbicides) of varying combinations in regions with intensive agriculture (Verderame and Scudiero 2019b). The number of studies using lizards to evaluate organic contaminants and their effects increased in the last decade. There were nineteen studies found in this review using lizards as experimental models or as field biomonitors. The lizard family most studied for organic compounds was Lacertidae, representing 58% of the total studies (Table 1). The species of this family found in the studies were *Podarcis sicula* (27% of the studies),

**Table 1** Studies that used lizards as experimental models to evaluate environmental contaminants (2009–2019) recorded during this review and characterized according to the family to which they belong, continent of origin, reported contaminant and author(s) of the work

Families	Continent	Organic contaminant	Inorganic contaminant	Authors
Agamidae	Asia	–	01	Al-Johany and Haffor (2009)
Cordylidae	Africa	–	01	Mcintyre and Whiting (2012)
Dactyloidae	North America	01	–	McMurry et al. (2012)
Iguanidae	South America	01	–	Cabarcas-Montalvo et al. (2012)
Lacertidae	Africa, Asia and Europe	11	06	Márquez-Ferrando et al. (2009), Marsili et al. (2009), Simoniello et al. (2010), Al-Hashem (2011), Capriglione et al. (2011), Amaral et al. (2012a, b), Bicho et al. (2013), Cardone (2015), Chang et al. (2016), Mingo et al. (2016), Verderame et al. (2016), Mingo et al. (2017), Nasri et al. (2017), Panitskiy et al. (2017), Márquez-Ferrando et al. (2009), Sargsyan et al. (2018), Simonyan et al. (2018), and Verderame and Scudiero (2019b)
Phrynosomatidae	North America	01	–	Aguilera et al. (2012)
Scincidae	Africa and Oceania	01	01	Soliman (2012) and Carpenter et al. (2016)
Teiidae	South America	02	–	Schaumburg et al. (2016) and Mestre et al. (2019)
Tropiduridae	South America	–	01	Salvador et al. (2018)
Varanidae	Africa	02	01	Ciliberti et al. (2011), Ciliberti et al. (2012), and Ciliberti et al. (2013)

*Podarcis muralis* (11%), *Podarcis bocagei* (11%), *Eremias argus* (5%), and *Acanthodactylus scutellatus* (5%) (Marsili et al. 2009; Al-Hashem 2011; Capriglione et al. 2011; Amaral et al. 2012a, b; Bicho et al. 2013; Cardone 2015; Chang et al. 2016; Verderame et al. 2016; Mingo et al. 2016, 2017; Verderame and Scudiero 2019b). However, it should be noted that most studies regarding the effects of organic contaminants on lizards are experimental.

The organic contaminants found in the recorded studies are listed in Table 2; most of them were tested agrochemicals (fungicides, insecticides, and herbicides), and most of them are toxic to the studied species. In these studies, exposure was orally acute at different levels. Biomarkers were used for the analyzes, which depict multiorganic changes, both morpho-histological involving the liver, kidneys, testicles, thyroid, adipose tissue, and changes at the systemic level of several parameters, including enzymatic activity, hormonal levels, and biochemical indicators (Marsili et al. 2009; Bicho et al. 2013; Cardone 2015; Chang et al. 2016; Mingo et al. 2016; Schaumburg et al. 2016).

The sublethal effects of pesticides on lizards have a wide variety of implications for exposed individuals such as hormonal changes, enzyme responses, oxidative stress, neurotoxic implications, immunosuppression, and also include physiological reactions such as feverish responses, fertility problems, development and locomotor performance (Amaral et al. 2012a, b; Bicho et al. 2013; Cardone 2015; Carpenter et al. 2016; Schaumburg et al. 2016).

In addition to agrochemicals, components of oil, coal, and explosives were also evaluated to identify the toxicity degree of these compounds in lizards (Marsili et al. 2009; Al-Hashem 2011; Capriglione et al. 2011; Cabarcas-Montalvo et al. 2012; Ciliberti et al. 2013; Cardone 2015; Chang et al. 2016; Schaumburg et al. 2016). Among the organic contaminants, glyphosate has been the main focus of studies, which assess the degrees of toxicity, as it is one of the most widely used herbicides worldwide with its main indication being in soy monoculture, despite this agrochemical having several negative effects in exposed biota (Carpenter et al. 2016; Mingo et al. 2016; Schaumburg et al. 2016; Mingo et al. 2017; Mestre et al. 2019; Verderame and Scudiero 2019b).

The effects of the glyphosate-based herbicide Roundup (RU) tested in sublethal concentrations found in these studies were genotoxic but not teratogenic,

inducing DNA damage in lizards popularly known as tegus (*Salvator merianae*), also being able to interfere in embryo development and hinder the reproduction of this species in areas exposed to these agrochemicals. Comet, micronuclei, and nuclear abnormality tests were used for this evaluation, demonstrating a significant increase in induced DNA damage for all concentrations above 100 µg RU/egg (Schaumburg et al. 2016).

Roundup Full II, Chlorpirifos Nufarm, and Cypermethrin Atanor contaminants in tegus eggs were also tested in experimental and subchronic conditions (Table 2), using concentrations equivalent to those recommended for application in crops for 3 months. The leukocyte count did not show significant differences between the control and treatment groups; however, it showed a slight negative trend in relation to organisms exposed to pesticides. Even at levels considered low, it was observed that these pesticides can cause immunotoxicity for *Salvator merianae*, including the application of these compounds in areas, which this species inhabits can affect its immune system. Corticosterone can decrease or increase in the presence of a stressor, and higher levels of corticosterone were found in lizards exposed to pesticides compared to the control group in this study (Mestre et al. 2019).

Further regarding glyphosate, another study evaluated its effects on the enzymatic activities of *P. muralis* lizards exposed in the field to the herbicide before and after application days of the contaminant in the agricultural area. The study showed there were strong signs of oxidative stress after collecting and analyzing the saliva of these animals (Mingo et al. 2017). The analyzed parameters were cholinesterase acetylcholinesterase activity (AChE), glutathione-S-Transferase (GST), and glutathione reductase (GR) activity. The results demonstrated neurological effects with a 5 to 30% decrease in acetylcholinesterase activity, with no influence on liver activity due to the lack of other biomarkers (Mingo et al. 2016). This same author published an evaluation of several other fungicide-type contaminants, in addition to glyphosate (Mingo et al. 2016; Table 2). As there was an inconclusive result as to what or which contaminants had been absorbed by the lizards, a new study was carried out with a focus on glyphosate, showing that oxidative stress was the main effect in both cases. An innovation of the authors in both studies was the less invasive collection technique for the animals by using swabs to detect the presence of toxic agents in the lizards' saliva. This technique had a success rate of

**Table 2** Organic compounds studied in lizard species as experimental models for assessing environmental contaminants (2009–2019) and their effects recorded during this review, characterized according to the classification of the compound, family, and species of lizard to which they belong, exposure time and route, verified effects, and author(s) of the work

Organic compound	Classification	Family/Species	Concentration of the substances	Exposure time	Exposure route	Effects	Authors
Polycyclic aromatic hydrocarbon (PAHs)	Petroleum compounds	Lacertidae <i>Podarcis sicula</i>	–	–	–	Presence of PAHs in lizard bile	Marsili et al. (2009)
Tar	Petroleum compounds	Lacertidae <i>Acanthodactylus scutellatus</i>	–	–	–	Damage of liver tissue cells and apoptosis	Al-Hashem (2011)
Thiophanate-methyl	Fungicide	Lacertidae <i>Podarcis sicula</i>	100 ml of 1.5% TM (1.5 g TM in 100 ml of water)	15, 30 and 40 day	Dermal spraying	Genotoxic effects	Capriglione et al. (2011)
Organophosphates, carbamates, and pyrethroids	Insecticides	Phrynosomatidae <i>Sceloporus serrifer</i> <i>Sceloporus torquatus</i>	–	–	–	No effects were detected	Aguilera et al. (2012)
Alachlor, Bentazone, Dicamba, Dimetenamide-P, Mesotrione, and Terbutylazine	Herbicide	Lacertidae <i>Podarcis bocagei</i>	–	–	–	Loss of body condition and histopathological changes	Amaral et al. (2012a, b)
Coal	*	Iguanidae <i>Iguana iguana</i>	–	–	–	Genotoxic effects	Cabarcas-Montalvo et al. (2012)
DDD, DDE, and Malathion	Insecticide	Varanidae <i>Varanus niloticus</i>	–	–	–	Low concentrations and no direct effects	Ciliberti et al. (2012)
Octa-hidro-1,3,5,7-tetranitro 1,3,5,7-tetrazocine (HMX)	Explosive	Dactyloidae <i>Anolis carolinensis</i>	HMX at 2000 mg/kg 2000 µg/g	14 days	Oral gavage, acute toxicity	No toxic effects observed	McMurry et al. (2012)
Alachlor, Bentazone, Dicamba, Dimetenamide-P, Mesotrione, and Terbutylazine	Herbicide	Lacertidae <i>Podarcis bocagei</i>	–	–	–	Disruptive effects of the thyroid	Bicho et al. (2013)
DDT and CPF	Insecticide	Varanidae <i>Varanus exanthematicus</i>	4 mg of DDT/kg <sup>-1</sup> and 0.5 mg of CPF/kg <sup>-1</sup> 4 µg/g and 0.5 µg/g	6 weeks	Oral, sub-chronic exposure	There was a high tolerance to the analyzed pollutants	Ciliberti et al. (2013)
Imidacloprid	Insecticide	Lacertidae <i>Podarcis sicula</i>	–	–	–	Toxic effects on the reproductive system	Cardone (2015)
	Herbicide	Scincidae					

Table 2 (continued)

Organic compound	Classification	Family/Species	Concentration of the substances	Exposure time	Exposure route	Effects	Authors
Glyphosate (Agpro Glyphosate 360 and Yates Roundup Weedkiller)		<i>Oligosoma polychroma</i>	144 mg of glyphosate for 1 L of water 144,000 µg/L	Single dose	Dermal route, indirect form	No significant effects were detected	Carpenter et al. (2016)
Lambda cyhalothrin	Insecticide	Lacertidae <i>Eremias argus</i>	10 mg kg of 1 pc of LCT 10 µg/g	1, 3, 6, 12, 24, 72, 120, and 168 h	Oral route	There was no bioaccumulation	Chang et al. (2016)
Glyphosate, Metrafenone, Metiram, Fosetyl-Al, Fluopicolide, Imintium, Difenconazole, Ciflutefnamide, Folpet, Quinoxifen, Penconazole, Miclobutanil, Fenhexamide, and potassium phosphonate	Herbicide and fungicides	Lacertidae <i>Podarcis muralis</i>	–	–	–	Oxidative stress	Mingo et al. (2016)
Glyphosate (Roundup)	Herbicide	Teiidae <i>Salvator merianae</i>	50–1600 µg of RU/egg	60 days	Egg shell	Genotoxic effects	Schaumburg et al. (2016)
Animal manure	Natural fertilizers	Lacertidae <i>Podarcis sicula</i>	–	–	–	Estrogenic contamination affects the reproductive system of lizards	Verderame et al. (2016)
Glyphosate	Herbicide	Lacertidae <i>Podarcis muralis</i>	–	–	–	Oxidative stress and genotoxic effects	Mingo et al. (2017)
Glyphosate (Roundup Full II), Atanor cypermethrin, and Nufarm chlorpyrifos	Herbicides and insecticide	Teiidae <i>Salvator merianae</i>	GLY 2% (a.p. 66.2%); CPF at 0.8% (a.p. 48%) and CYP at 0.12% (a.p. 25%)	3 months	Dermal route, indirect form	Effects on the immune system	Mestre et al. (2019)
Glyphosate	Herbicide	Lacertidae <i>Podarcis sicula</i>	0,05 e 0,5 µg/kg pc em 50 µl de água	3 weeks	Oral gavage	Xenoeostrogenic action on the liver	Verderame and Scudiero (2019b)

around 90% (Mingo et al. 2016), therefore being highly recommended to reduce environmental impact on animals, especially those which are threatened with extinction by the IUCN red list.

An association was found in New Zealand between two commercial glyphosate formulations (Agpro Glyfosate 360 and Yates Roundup Weedkiller) and the thermoregulation behavior of *Oligosoma polychroma* lizards. The lizards were divided into three groups for the experiment, one control and one for each glyphosate formulation; the treatment occurred only once, simulating the application in the field. The treatment groups were covered with straw and sprayed with the herbicide at a concentration of 144 mg of glyphosate to 1 L of water; this concentration was considered low within the range, which can be used to control weeds. The animals were weighed during the 4 weeks before treatment and 4 weeks after treatment, but there was no evidence that the exposure influenced their weight. Exposure to the Yates Roundup Weedkiller formulation was correlated with a search for warmer temperatures by the lizards, more than in the other two tests. Still regarding thermoregulatory behavior, it was observed that the animals presented a feverish reaction looking for warmer areas of the terrarium in an attempt to minimize the stress caused by herbicides (Carpenter et al. 2016). However, no significant effects were identified; a posteriori effects may appear, but nothing occurred during the study period. Despite this, the authors suggest more transparency regarding the compositions of the commercialized agrochemicals, so that it is possible to know the toxicity level of their active ingredients more than their formulations (Carpenter et al. 2016).

In a study with male *P. sicula* lizards found in areas of organic farms free from pesticides and similar chemical groups, it was found that they had estrogenic contamination after histological and molecular analyzes of their livers; biosynthetic changes were found in their liver tissues due to the presence of the vitelogenin biosynthesis gene (VTG) in males, which is considered a biomarker of estrogen contamination (Verderame et al. 2016). Even in areas free from agrochemicals, this result demonstrates that the use of substances considered natural by farmers can also indirectly affect the fauna that resides there. Verderame et al. (2016) also add that the use of natural fertilizers such as animal manure can generate an accumulation of natural steroids, which end up interfering in the reproduction of lizards of this species, reinforcing the described speculations.

Other pesticides have also been evaluated using lizards; contamination by the thiophanate-methyl fungicide causes genotoxic effects in Italian *P. sicula* lizards, producing high frequencies of DNA damage, as well as enabling the increase in the number of micronucleated cells, leading to the conclusion that this contaminant must accumulate in the organism, inducing chromosome instability by interfering with the formation of microtubules, resulting in an aneugenic effect (Capriglione et al. 2011). Comet (frequency of DNA damage), micronuclei (frequency of cells with micronuclei), and chromosomal abnormality (arm breaks and Robertson fusions) tests were used for this evaluation.

Cardone (2015) used hormonal and morphological analysis techniques to assess oral exposure of *P. sicula* lizards to the insecticide Imidacloprid, finding that this compound consistently affected the lizards' reproductive system. Pure glyphosate administered orally was considered toxic to the liver of this same species, even exerting a xenoestrogenic action, with histological and biomolecular analyzes of the animals' liver being performed for this experiment. The liver structure in the control group animals was considered regular, whereas few degranulation areas were found in the liver parenchyma for the animals receiving the lowest doses of glyphosate treatments, while the liver parenchyma was more damaged with greater degranulation areas for the highest dose, in addition to edema in blood vessels and bile ducts (Verderame and Scudiero 2019b).

For the *P. bocagei* species, studies carried out in Portugal evaluated the risk of exposure to various herbicides in natural field conditions (Table 2) in comparing areas without the influence of these compounds with contaminated areas, verifying that animals exposed to this type of contaminant presented a more precarious health condition than individuals in the control areas; one of the animals in the exposed area even contained an intestinal parasite (Amaral et al. 2012b). In addition, the most significant presence of hematoparasites was found in contaminated areas, reaching 18% higher than the reference areas. The animals showed histopathological changes such as fibrosis and degeneration of hepatocytes, as well as disruptive effects of the thyroid gland, which is a problem that can affect the lizard's reproductive system, with the alachlor compound being considered responsible in the latter case (Amaral et al. 2012b; Bicho et al. 2013).

Liver tissue cells from *Acanthodactylus scutellatus* lizards were damaged, and several of them underwent apoptosis in areas contaminated with tar in an oil field in Kuwait, confirming that prolonged exposure to oil-based contaminants causes severe pathologies to animals living in those areas; histological analyzes were performed for this evaluation (Al-Hashem 2011). However, in a study conducted in oil fields in Italy, *Podarcis sicula* lizards exposed to high levels of polycyclic aromatic hydrocarbons (PAHs) and trace elements were evaluated, without any pathological effect being found in the individuals (Marsili et al. 2009). McMurry et al. (2012) carried out an experimental study with *Anolis carolinensis* lizards to evaluate the effects of the explosive octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocina HMX on these animals. A dose of 2000 µg/g of HMX was administered orally in this experiment, and the animals were initially observed at regular intervals, and later three times a day, at the end of the tests it was found that this substance did not present high toxicity for the adult green Anole lizards. However, there was a reduction in the hatching rate for the eggs in those exposed to soil contaminated with this explosive.

Coal mines are considered contamination sources for biota, given that coal is toxic to the respiratory system. The environmental impact in two cities close to coal mines in northern Colombia was assessed in a study on two animal species, *Mus musculus* (rat) and *Iguana iguana* (lizard). There was an increase in the frequency of damage to DNA and cells with micronuclei of the biomonitors used in impacted areas near the mine, with the consequence of genotoxic effects linked to the contamination degree of these environments (Cabarcas-Montalvo et al. 2012).

An important point to note is the variability of responses described on the consequences of organic contaminants in lizards and their relationship with both toxicokinetic and toxicodynamic aspects, facts that have an impact on the response degree and the consequent usefulness of each species as a biomonitor. For example, some studies have indicated no bioaccumulation or visible intoxication for the tested contaminants, as in the case of pyrethroids in the Chinese *Eremias argus* lizards. The explanation for this is the differences in the bioaccumulative capacity, resulting in a more efficient metabolism, decreasing the toxic capacity of substances due to a higher metabolic rate of toxic substances by the liver (Chang et al. 2016). In a study to define biomarkers

for two species of lizards, Aguilera et al. (2012) used pieces of tail from the *Sceloporus serrifer* and *Sceloporus torquatus* lizards at two locations in Mexico, an environmental protection area and another highly polluted area in an industrial park, to analyze enzymatic activities in the presence of organophosphate, carbamate and pyrethroid pesticides, but the animals did not show evident effects.

Another possibility raised by Ciliberti et al. (2012, 2013) in this type of result is that the lizards may have a high tolerance to the pollutants in question or there is not such a severe environmental contamination, as was the case with Savannah monitors (*Varanus exanthematicus*) and Nile monitors (*Varanus niloticus*), respectively. In the first case, the animals appeared capable of handling high contamination by organochlorines and lead derived from pesticides in the environment. In this scenario, it is also worth considering the size of the animal, which has a greater body mass.

#### Inorganic contaminants

There has been significant advancement in scientific studies using lizards as bioindicators of heavy metals and radionuclides over the past 17 years, considering the last review carried out by Campbell and Campbell (2002) as a reference when few studies on this topic were found and only two addressed the effects of heavy metals in this zoological group.

In addition, there is concern to verify the bioaccumulation of metals in the organs to confirm the usefulness of the lizard as a bioindicator, despite there being discontinuity in studies to assess the real effects on lizards; most studies do not actually show the pathological effects of contamination on animals. McIntyre and Whiting (2012) studied the increased rate of heavy metal concentration in endemic lizards (*Smaug giganteus*) in two gold mining areas in South Africa and argued that there is still little understanding about the bioaccumulative effects of these contaminants, but one of the factors is a decrease in the individual's body condition, even when there is a high availability of prey in the environment. The study areas were two mining areas and two control areas. Significant differences were found between the sampled locations in the sodium (Na), nickel (Ni), aluminum (Al), chromium (Cr), iron (Fe), manganese (Mn), silicon (Si), and sulfur (S) concentrations in lizard whole blood samples, with samples from the mine



area always presenting higher values. The metals, which showed the most significant values in the statistical tests, were lithium (Li) with 9.7 µg/g, nickel (Ni) with 7.45 µg/g, copper (Cu) with 5.58 µg/g, and chromium (Cr) with 5.42 µg/g, which may be the metals responsible for the aforementioned effects. The lizards used in this study are on the IUCN list of endangered animals, classified as vulnerable, so less invasive methods were used and the animals did not die. They were marked with passive transponders integrated with an alphanumeric code, injected subcutaneously in the post-femoral region of the animal.

In the ten studies that addressed heavy metals in lizards published in the last decade (Table 3), the concentrations of metals were analyzed in various organs and tissues, with blood, liver, kidneys, lungs, stomach, and tail tip being the most common (Al-Johany and Haffor 2009; Márquez-Ferrando et al. 2009; Simoniello et al. 2010; Ciliberti et al. 2011; McIntyre and Whiting 2012; Soliman 2012; Nasri et al. 2017; Salvador et al. 2018; Sargsyan et al. 2018; Simonyan et al. 2018). Among these, an innovative study evaluated the presence of metals in the helminths that parasitized *Chalcides ocellatus* lizards in three areas (industrial

**Table 3** Inorganic compounds studied in different species of lizards and their effects

Organic compound	Classification	Family/Species	Effects	Authors
Cadmium (Cd)	Heavy metal	Agamidae <i>Urematix aegyptius</i>	Cadmium-induced pneumothelial and endothelial injury	Al-Johany and Haffor (2009)
Mercury (Hg), chromium (Cr), antimony (Sb), copper (Cu), thallium (Tl), cadmium (Cd), lead (Pb), tin (Sn), barium (Ba), strontium (Sr), manganese (Mn), rubidium (Rb), astate (As), and zinc (Zn)	Heavy metals	Lacertidae <i>Psammotromus algirus</i>	All metals bioaccumulated on the individuals' tails.	Márquez-Ferrando et al. (2009)
Cadmium (Cd)	Heavy metal	Lacertidae <i>Podarcis sicula</i>	Rupture of the hepatic matrix	Simoniello et al. (2010)
Cadmium (Cd) and lead (Pb)	Heavy metals	Varanidae <i>Varanus niloticus</i>	There was no bioaccumulation in the tissues.	Ciliberti et al. (2011)
Nickel (Ni), aluminum (Al), copper (Cu), chrome (Cr), sulfur (S), iron (Fe), tungsten (W), lithium (Li), sodium (Na), silicon (Si), manganese (Mn), and bismuth (Bi)	Heavy metals	Cordylidae <i>Smaug ginganteus</i>	Decreased body condition	McIntyre and Whiting (2012)
cadmium (Cd), lead (Pb), and nickel (Ni).	Heavy metals	Scincidae <i>Chalcides ocellatus</i>	There was bioaccumulation of metals in the cestode parasites present in the lizards.	Soliman (2012)
Cadmium (Cd), zinc (Zn), and lead (Pb)	Heavy metals	Lacertidae <i>Acanthodactylus boskianus</i>	Bioaccumulation of the three metals in lizard tissues	Nasri et al. (2017)
<sup>137</sup> Cs and <sup>90</sup> Sr	Radionuclides	Lacertidae <i>Lacerta agilis</i>	–	Panitskiy et al. (2017)
Aluminum (Al) and zinc (Zn)	Heavy metals	Tropiduridae <i>Tropidurus torquatus</i>	Bioaccumulation of zinc in fat and aluminum in the liver	Salvador et al. (2018)
Chromium (Cr), cobalt (Co), copper (Cu), zinc (Zn), astate (As), molybdenum (Mo), cadmium (Cd), lead (Pb), and nickel (Ni)	Heavy metals	Lacertidae <i>Darevskia armeniaca</i> ; <i>Darevskia raddei</i>	Genotoxic and epigenetic effects	Sargsyan et al. (2018)
Chromium (Cr), cobalt (Co), copper (Cu), zinc (Zn), astate (As), molybdenum (Mo), cadmium (Cd), and lead (Pb).	Heavy metals	Lacertidae <i>Darevskia armeniaca</i> ; <i>Darevskia raddei</i>	Genotoxic effects	Simonyan et al. (2018)

area, rural area, and urban area) in Egypt. In this study, bioaccumulation of the metals cadmium, lead, and nickel was found in cestode parasites present in animals (Soliman 2012). A number of metals and other inorganic compounds were evaluated in this study, with the main ones being: lead (Pb), cadmium (Cd), chrome (Cr), aluminum (Al), zinc (Zn), copper (Cu), iron (Fe), and mercury (Hg).

Some studies have found a correlation between the ingestion of prey contaminated with heavy metals and the bioaccumulation of these metals in lizards (Márquez-Ferrando et al. 2009; Nasri et al. 2017; Salvador et al. 2018). One of these studies registered contamination of the local biota by heavy metals in the green corridor of Guadiamar (Spain) after the collapse of a mine tailings tank, even after 8 years of this fact. The *Psammodromus algirus* lizards, which inhabited the impacted area, had high concentrations of heavy metals in their tissues, with the main contamination route being from ingesting polluted prey. Considering that this lizard is a generalist and feeds on invertebrates, it can be said that the entire local biota is contaminated (Márquez-Ferrando et al. 2009). In Tunisia, Nasri et al. (2017) evaluated the accumulation of cadmium, zinc, and lead in lizards of the *Acanthodactylus boskianus* species, which live near a phosphate treatment plant, finding a correlation between ingesting prey contaminated with metals and the bioaccumulation of these metals in lizards; a fact which led to the conclusion that there was a transfer of contaminants from aquatic to land networks. The concentrations were analyzed in the liver, kidneys, stomach, and tail pieces, and it was found that cadmium metal was found in greater concentration in the liver, and lead in the animals' tails (Nasri et al. 2017).

There is also the possibility of ingesting contaminated sediment particles along with the food, with larger individuals having the capacity to accumulate larger amounts of pollutants; however, the longevity of the species must also be considered. A study carried out in Saudi Arabia showed that long-term exposure (by ingestion) to the cadmium element generates structural pathologies in the lung cells of the *Uromastix aegyptius* lizard due to an elevation of reactive oxygen species. The experimental group was submitted to ingestion of cadmium mixed with the diet (200 mg/g) for 7 weeks, and then the animals were sacrificed; blood samples were subsequently collected by cardiac puncture, and the analysis was performed by leukocyte count and the

isolated lungs by ultrastructural analysis (Al-Johany and Haffor 2009). In an experimental study in Naples, Italy, with *Podarcis sicula*, Simoniello et al. (2010) evaluated the lizard's response to cadmium metal liver poisoning and concluded that cadmium ingestion destabilizes the liver tissue structure of these lizards. The animals were divided into a control group, received only food, and the experimental group received a single intraperitoneal cadmium dose of 10 mg/10 g of body weight.

Two of the studies analyzed used techniques at the molecular level (comet test, micronucleus test, and global DNA methylation) to assess DNA damage caused by exposure to heavy metals and found a clear relationship between heavy metal contamination and an increase in damage frequency to DNA and micronucleus cells in lizards (*Darevskia armeniaca* and *Darevskia raddei*), which were sensitive to pollutants. These studies were carried out in Armenia in seven different areas covering a protected area, industrial and urban areas (Sargsyan et al. 2018; Simonyan et al. 2018). It was also assessed whether the age of individuals would influence the tests, but it was found that lizards of the *Darevskia* genus have a greater capacity to cope with stresses generated by xenobiotics with an increase in their life span; therefore, the tests were not affected by age (Sargsyan et al. 2018).

The only study in radionuclide areas was conducted at a nuclear weapons testing center in Kazakhstan (Panitskiy et al. 2017). In this study, analyzes of the amount of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  radionuclides were performed in the bodies of *Lacerta agilis* lizards of the Lacertidae family; however, as also observed in most studies with heavy metals, the effects of these radioactive compounds on lizards were not presented, only the form with which radioactive chemical elements are redistributed in the environment through animals.

As pointed out by Panitskiy et al. (2017), there is a lack of experimental data on the evaluation of radionuclide transfer parameters in reptile bodies. In addition, studies with reptiles in areas of natural radiation were also not found during this review, which is one of the main points to be advanced in research worldwide.

A very relevant contribution presented by Ciliberti et al. (2013) was the use of the tip of the lizards' tail for studies on lead contamination, considering that this tissue is a good bioindicator of this element and also reduces the environmental impact. Nasri et al. (2017) agrees and reinforces this statement, indicating the liver as an aluminum bioaccumulator and animal fat as a zinc

bioaccumulator; however, both did not describe the real effects of these metals on the lizards' organism. Therefore, it is extremely important that studies are carried out which describe possible pathological effects in these animals.

Therefore, it was found in this review that most studies on environmental contaminants are experimental. However, field studies can more clearly show and evidence the environmental effects of such contaminants, considering all the variables to which animals are exposed in nature.

## Conclusion

This review on environmental contaminants and biomonitoring by lizards made it possible to verify that most studies are focused on organic contaminants, specifically agrochemicals, such as herbicides, fungicides, and insecticides. In addition, it is noteworthy that the studies are mostly experimental, and it should also be noted that the consequences in areas exposed to a risk situation can be much worse for lizards. Still, it is worth noting that studies on the evaluation of organic contaminants are those which best express the effects of toxic elements/substances on lizard organisms. This is an important point, which still needs to advance in studies on the action of inorganic contaminants such as heavy metals in lizards. Despite the gaps and deficiencies found, this study area is recent and has been growing in recent years. Biomonitoring of chemical agents in itself still has a long way to go in order to have a better understanding of the action of environmental pollutants and the redistribution processes in the environment, and therefore to discuss and apply methodologies which reduce these impacts on nature.

Although there are still few studies which show the pathological effects of inorganic contaminants in lizards, most validate these animals as bioindicators and discuss, which organs are better biomarkers. Among the contaminants discussed, studies on areas rich in radiation and radionuclides are the least studied. On the other hand, it became clear throughout this review that lizards are ideal models for ecotoxicological studies. These facts fully justify the use of lizards for biomonitoring regions with extreme conditions such as the Brazilian semi-arid region. In addition, studies involving native animals from regions with natural radioactivity have demonstrated the usefulness such as in the radioisotope

biomonitoring, similar to metal contamination, which can predict the resulting impact on other organisms, including human health.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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