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Chapter

Metabolic Impairments Caused by Pesticides in Mammals and Their Interactions with Other Pollutants

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Abstract

The biological systems are exposed to a complex environment in which the contaminants can interact in a synergistic/antagonistic fashion and for this reason, the study of "chemical cocktails" is of great interest to fully understand the final biological effect. To evaluate the final biological response of a pollutant, it is essential to have an adequate analytical methodology that allows the correct monitoring of environmental systems in order to establish their quality, and, when appropriate, the application of corrective measures. Undoubtedly, massive methods "the omics" are among the most efficient current tools. To this end, transcriptomics, proteomics, metabolomics and chemical speciation can provide very useful information, mainly when they are combined. However, the combination of proteomics with metabolomics has some drawbacks as the temporal space is different (i.e. metabolomics gives information about what happens right now, but it can be related with numerous post-translational modifications happened previously). In this sense, it seems that the combination of genomics with metabolomics is easier. Thus, when metabolomics data are interpreted in combination with genomic, transcriptomic and proteomic results, in the so-called systems biology approach, a holistic knowledge of the organism/process under investigation can be achieved.

Keywords: pesticides, mammals, metabolomics, metals, speciation, omics

1. Introduction

The evaluation of the biological response in living organisms against environmental pollution requires the use of environmental bioindicators or laboratory models of increasing complexity (mollusks, crustaceans and rodents) [1, 2]. A mammal should be always included to integrate the diverse biological filters present in humans, like the digestive tract, which regulates the passage of contaminants to be later distributed through the bloodstream to other parts of the body [3]. In

addition, cell cultures are also important in these types of studies, especially to translate the effects of pollutants to humans. The use of cell cultures also allows performing many experiments without the difficulties of the experimentation with animals [4].

Until now, there has been a great concern, mainly relying on individual environmental pollutants, to study their potential risks. However, the evaluation of health effects of chemicals by considering data obtained just from a single chemical, leads to over or underestimates the joint toxicity. In this sense, studies concerning the combined effects of pollutants better than the toxicity assessment of single chemicals, reflect the realistic impact of environmental exposures [5]. Thus, the experimentation with animal models to evaluate the biological response against environmental pollutants, and their possible translation to the effects in humans, should be carried out designing experiments that mimic as much as possible the environment. To this end, controlled exposure experiments to "chemical cocktails" that integrate several environmental pollutants of different chemical groups can be a good approximation. Although the design of experiments is significantly more complex, the obtained information is of great interest to evaluate the real effects of contaminants in the environment [6].

On the other hand, the joint toxicity of chemical mixtures can be independent one from each other (additive), stronger (synergistic) or weaker (antagonistic), depending of on the sum of effects from individual exposures or not [7].

The biological response can be evaluated using model organisms, but also with free-living species that can also serve as biomarkers of environmental pollution. Although the use of free-living organisms allows evaluating the biological response taking into account all the existing environmental factors and their interactions, and for this reason is closer to the reality, the main pitfalls are related with the difficulty to isolate the metabolic responses connected with a particular pollutant [8]. Otherwise, the study with model organisms is easier and cheaper, but requires a qualified technician to administrate single xenobiotics or their cocktails and the results can be biased by the selected administration route (e.g., subcutaneous injection), model organism used, etc., [10]. Nevertheless, the study of the biological response in free-living animals is of great interest to validate the results obtained in the exposure experiments with model organisms [11, 13].

2. Interactions of metals and chemical species through antagonistic and synergistic mechanisms

As previously commented, biological systems are exposed to a complex environment in which contaminants can interact by means of antagonistic or synergistic mechanisms making mandatory global studies (i.e.,-omics) to evaluate the biological response with an holistic view [14]. In connection to that, selenium has been claimed as the most important element regarding its antagonistic protective action against numerous contaminants (i.e., pesticides and metals). Likewise, it has been stated that cardiovascular damages caused by mercury can be antagonized by selenium [15], but also the neurotoxicity [16] and renal toxicity [17] caused by this element. Moreover, selenium present also protective character against skin cancer induced by arsenic exposure [18], chromosomal aberrations induced by cadmium [19], oxidative stress and lipid peroxidation caused by organophosphorus pesticides [20]. Moreover, the chemical form or specie of selenium is very important since the essentiality or toxicity is directly related to that. Likewise, some of them, especially selenoproteins are peroxidases or oxidoreductases, which protect against oxidative stress [21–24, 26], while inorganic selenium is toxic at high levels [14]. Our research

Mammal	Interaction	Biological effect	References
Interactions of selenium- and arsenic containing species			
Humans	A S_{etotal}/A_{stotal}	DNA hypomethylation/cancer	[39]
Humans		Se reduces the risk of As-related skin lesions and cancer	[16, 40, 41]
Rats	2−A SeO_3/iAs(III)	As prevents lethal liver damage caused by Se	[42]
Rats		As prevents Se-induced cataracts	[43]
Humans		Skin lesion/skin cancer	[44]
Mice		As prevents carcinogenic effect of Se	[45]
Rats		As prevents carcinogenic effect of Se	[46]
Rats		As protects against the toxicity of Se (growth, mortality rate, pathological condition of the liver)	[47–50]
Rats		As induces mucosal glutathione synthesis, explaining its protective effect against Se	[51]
Dogs		As antagonizes Se-induced subnormal growth and restricted food intake	[52]
Cattle		As protects against Se toxicity	[47, 53]
Hogs		As protects against Se toxicity	[48]
Steers		As protects against Se toxicity	[54]
Mice		Se prevents As-induced cytotoxicity	[55]
Mice	2 − 3−A SeO_3/ AsO_4	Se decreases the ratio of organic/inorganic As	[56]
Hamsters		Se decreases As methylation	[14]
Rats	S SeBet/iAs(III)	Coadministration enhances the tumor-suppressive effect of Se	[13]
Interactions of selenium- and mercury-containing species			
Humans	A Setotal/Hgtotal	Se prevents Hg induced cardiovascular diseases	[15]
Humans	A Setotal/$MeHg^+$	Se inhibits Hg-induced neurotoxicity	[57]
Humans		Se inhibits Hg-induced cardiovascular diseases	[58]
Rats		Se may alter the reproductive and developmental toxicity of MeHg+	[59]
Rats	2−/2+ A SeO_3 Hg	Se antagonizes Hg-induced intestinal necrosis	[60]
Rats		Se prevents Hg renotoxicity	[62]
Mice		Se prolongs the half-lives of Hg-exposed animals	[63]
Rats		Se changes the subcellular Hg distribution	[64]
Mice	2−/+ A SeO_4 MeHg	Se protects against Hg-induced neurotoxicity	[65]
Rats	A SeMet/Hg^{2+}	Se inhibits the effects of Hg on organic activities	[66]
Rats	A SeMet/$MeHg^+$	Se prevents Hg-induced porphyrinuria	[67]
Humans	A SeProt/ Hg^0	Se detoxifies Hg	[68]
Mice	A SeProt/$MeHg^+$	Hg affects the activities of selenoenzymes	[69]
Interactions of selenium and sulfur-containing species			
Sheep	A SeMet/sulfur compounds	More Se is incorporated into wool and plasma protein when dietary S is limiting	[70]

Mammal	Interaction	Biological effect	References
Interactions of selenium with species of elements			
Rats	A Se/Sb	Sb has a partially protective effect against Se toxicity	[49, 52, 71]
Rats	A Se/Bi	Bi has a partially protective effect against Se toxicity	[49, 71]
Human cells	A Se/Cd	Se protects against Cd toxicity	[72]
Rats	A Se/Cd	Se prevents Cd-induced oxidative stress	[73]
	A Se/Cd	Se protects against Cd-induced nephrotoxicity and hepatotoxicity	[74]
Mice	A Se/Cd	Se protects against Cd-induced chromosomal aberrations	[19]
Monkeys	A Se/Cd	Se protects enzyme systems	[75]
Rats	S Se/Cd	Se and Cd affect the hepatic gluconeogenic pathway	[76]
	A Se/Cd	Se partially restores Cd-induced oxidative stress and decreased sperm count and motility	[77]
	A Se/Cd	Se antagonizes the Cd-induced inhibition of hepatic drug metabolism	[78]
	A Se/Cd	Se antagonizes Cd-induced testicular damage	[79]
	A Se/Cd	Hepatoprotective effects of Se against Cd	[80]
	A Se/Ge	Ge partially protects against Se toxicity	[49, 71]
	A Se/Ni	Se may antagonize the deleterious effects of Ni during reproduction	[81, 82]
Mice	S Se/Ag	Se protects against Ag-induced lipid peroxidation in the liver	[83, 84]
	S Se/Te/Hg	Hg retention is increased by pre-administration of Te or Se	[85]
Rats	A W/Se	W partially protects against Se toxicity	[49, 71]

Table 1.
Main interaction of selenium species with other elements.

group has been working with *Mus musculus* mice exposed to the pesticide dichlorodiphenyldichloroethylene (DDE) and selenium [27] to study the join effect in the metabolome, as well as several exposure experiments to one or two metals (metalloids) in *Mus musculus* like Se-Cd [2], Hg-Se [28] and others.

Perhaps, the most studied interaction of selenium is with mercury, which was first investigated in 1967 in rats exposed to mercury chloride and selenite [29]. One of the proposed mechanism for this interaction is the formation of Hg-Se complexes, which can result in an increased whole-body retention of Hg after the co-exposure to both elements [29, 30]. However, although this interaction is well known, the mechanisms related to that remain unsolved. It has been stated that inorganic mercury can be incorporated to selenoproteins, peptides and prosthetic groups of selenoenzymes, by reaction of mercury with the selenol group of selenocysteine (SeCys) [31]. The lower pKa of SeCys makes it more reactive that Cys and for this reason, the former reacts by means of -she with Hg with stronger affinity than -SH groups. Mercury can be also incorporated into selenoproteins which play important roles in the maintenance of cellular homeostasis [26, 32]. To this end, Hg^{2-} can react with Se^{2-} (selenides), selenols or hydrogen selenide to form ternary complexes Hg—Se—S together with glutathione that finally bond to

selenoprotein P (SelP) [32–34]. The dysfunction of several proteins, like selenoproteins is in the basis of several diseases like carcinogenesis. In this sense, selenoprotein P accounts for the highest content of selenium in serum and can be also present in other selenoenzymes such as thioredoxin reductase (ThxR), glutathione peroxidase (GPx) and selenoprotein P [35, 36].

Previous studies carried out in our research group using *Mus musculus* mice as a model organism exposed to mercury and selenium demonstrate that the levels of selenoprotein P increase in the liver (extracts from hepatic cytosolic extracts) and serum after Hg exposure, and that selenite supplementation increase the effect [28]. In this context, the synthesis of SelP from selenite increases after mercury exposure since this protein serves as a vehicle for Hg detoxification [37]. This fact is also in good agreement with the decrease observed in the selenometabolites found in serum and the correlative increase in liver, where SelP synthesis takes places from selenite to be later transferred to bloodstream [38]. Moreover, our studies demonstrated that the accumulation of SelP in the liver is higher when the diet is supplemented with selenium. On the other hand, the concentration of selenoalbumin increase in liver and decrease in serum after Hg exposure to transport selenium for the synthesis of SelP [38]. Thus, these results demonstrate the interaction of mercury and selenium in the detoxification process induced by the later, the accumulation of selenoproteins in liver and bloodstream and the homeostasis of elements.

Table 1 shows the main selenium interactions with other metals in mammals.

3. Pesticides, metals and their impairments at molecular level

The joint effect induced by pesticides and metal ions increase, in general, the toxicity (synergism) like DNA damage, mortality rate and reduction in the reproduction rate, as well as changes in enzyme activities [86]. The exposure to environmental pollutants such as polychlorinated biphenyls (PCBs), organochlorine pesticides and heavy metals, has been associated to immunotoxic effects in mammals such as alterations of both the innate and adaptive arms of immune systems, which include aspects of cellular and humoral immunity [87].

Metabolic impairments caused by the join exposure of the pesticide DDE and selenium have been studied using a metabolomics approach [27]. In this study, we conclude that about 70 metabolites are altered in the most metabolically active organs, like liver and kidney, but also in brain, and that they are related with the pathways of oxidative stress, degradation of phospholipidic membrane, β-oxidation and energy metabolism, which confirm the potential of combined metabolomic platforms in environmental studies. Moreover, several metabolites present different response (increase or decrease against the control group) in the organs studied indicating a possible traffic between them. This is the case of liver and kidney, which are the most metabolically active organs and present five metabolites altered with the opposite tendency between them, namely: diacylglycerol (DAG) (18:2/18:1) and ornithine and triacylglycerols (TAG) (18:4/18:4/20:4), TAG (16:0/18:1/22:0) and TAG (18:4/18:4/22:6).

To deep insight into the protective effect of selenium against the toxic effect of DDE, we can compare mice supplemented with DDE, DDE + selenium and selenium against the control group. Selenium counteracts the effect of DDE on seven metabolites because they show a different response among the studied groups when they are compared against the control, which is illustrated in **Figure 1**. These metabolites can be used a biomarkers of the antagonistic interaction between selenium and DDE.

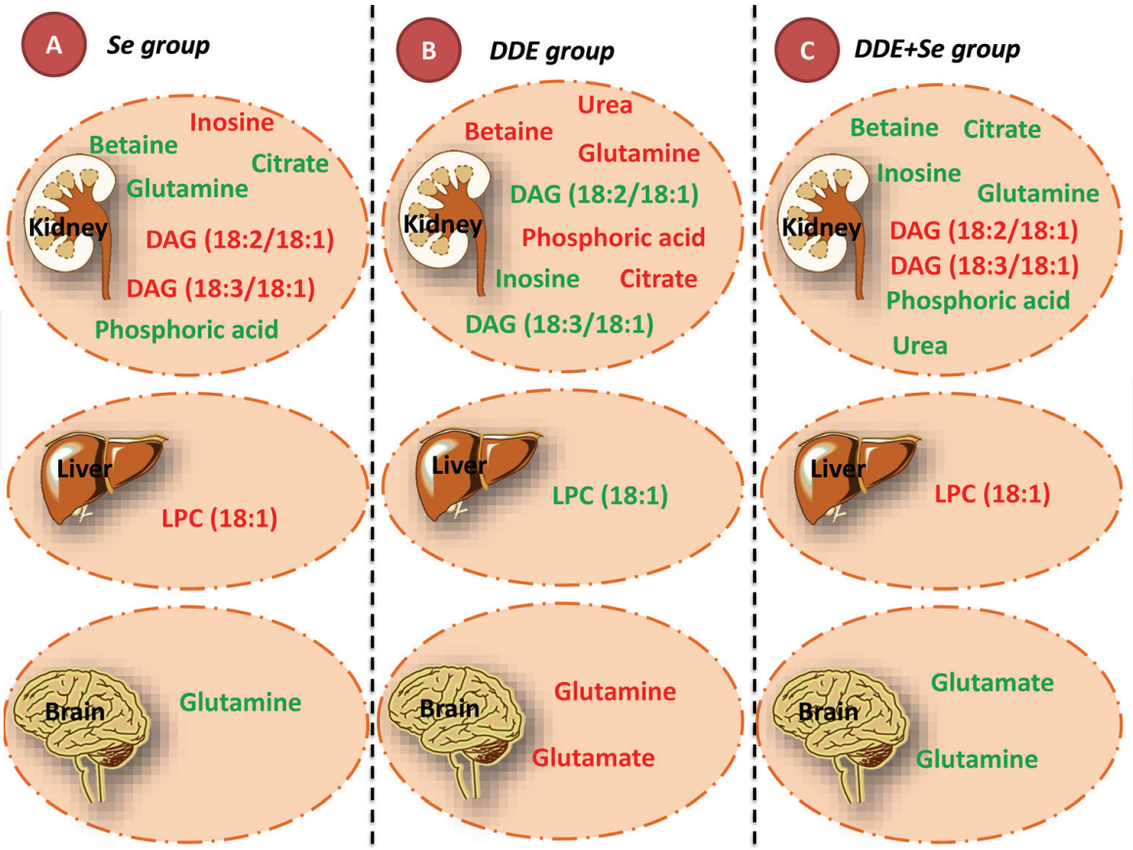

Figure 1.
Different response of metabolites in Mus musculus *mice exposed to: (A) selenium, (B) DDE, (C) selenium and DDE. Red word: Increased; Green word: Decreased.*

It is also remarkable that these antagonistic interactions between DDE and Se usually take place in kidney, since the majority of metabolites that present different response between the mice supplemented with DDE and DDE with Se occur in this organ.

4. Pharmaceutical active compounds, metals and pesticides and their impairments at molecular level

The high consumption of medicines, cosmetic products, as well as pesticides in modern agriculture or plastics in the handling and conservation of food, among others, has led to the appearance in the environment, mainly in soils and aquatic environment, of a series of compounds harmful to the living organisms that inhabit it. These are the well-known "organic micropollutants" (MCOs), a large group of substances that are continuously incorporated into the environment and that, in general, are difficult to eliminate. These substances are classified into two main groups: priority pollutants, and the so-called emerging pollutants (CE). Its detection in the environment has been possible, in many cases, thanks to the development of new and more sensitive analytical technologies that have allowed the detection of these compounds in all types of environmental samples, even in zones, apparently, not subjected to this "contaminant pressure". The analysis of "effluents of wastewater treatment plants" (WWTPs), urban and industrial, has shown, unequivocally, in general, very poor elimination of most of these substances, which is why an incorporation occurs continuously through this way to aquatic and terrestrial ecosystems [88].

Thus, besides metals and pesticides, pharmacologically active compounds (PAC), is a group of emerging contaminants, which are receiving increasing

attention because of their potential harmful effects for the environment and human health. The prevention of the emission of priority and emerging pollutants through wastewater treatment plants effluents into the aqueous environment requires the development of new treatment technologies that ensure the quality of receiving water bodies since the actual treatments are deficient and these substances are continuously incorporated into the environment [89]. Actually, analytical methodologies allow determining these substances in almost all samples and at very low levels [88, 90]. All the pollutants present in the aquatic and therefore terrestrial systems, as well as the products that are originated from them by degradation or metabolization, have an inevitable effect on the organisms that inhabit them, it can be highlighted, for example, their presence in coastal sediments or American red crab [90].

The studies related to the effect on biological responses by the presence of chemical cocktails concerning mixtures of pesticides, PACs and metals are scarce [91]. In this context, the effect that the presence of the antibiotic, ciprofloxacin has on the toxicity, distribution and accumulation of copper has been studied in earthworm (*Eisenia fetida*) [92]. However, the study of the biological response of mammals against "chemical cocktails" including metals, pesticides, and PACs has not been performed until now.

5. Omic methodologies to assess health effects of pesticides and other organic micropollutants

It is essential to have an adequate analytical methodology that allows the correct monitoring of environmental systems in order to establish their quality, and, when appropriate, the application of corrective measures. Undoubtedly, massive methods "the omics" are among the most efficient current tools. In this sense, it has been demonstrated that alterations of the homeostatic cycles are shown at the transcriptional level (transcriptomics) [93], by overexpression or inhibition of proteins (proteomics) [94] and by modifications of the metabolic cycles (metabolomics) [1, 95]. On the other hand, it has been stated that approximately 1/3 of proteins need the presence of metals as cofactors to develop their function (metalloproteins) and that metals influence on more than 50% of the proteins [96]. These metals play essential roles due to their catalytic properties or influence the structure of proteins and generally, the genome determines their presence in molecules [97]. Thus, metallomics allows understanding the distribution of elements, concentration at equilibrium of free metallic ions or free elements in a cellular compartment, cell or organism [98] and refers to the identity and/or quantity of metals/metalloids and their species [99]. Likewise, it has been proposed the integration of a global (holistic) view, much more realistic of the processes that takes place in the environment [95].

To this end, genomics decipher the information that determines cell function which is contained in the cellular core, transcriptomics reveals gene expression and proteomics make possible the examination of protein synthesis and cell signaling. On the other hand, in the establishment of transcriptional expression profiles that explain the gene function is critical in **environmental transcriptomics**, and the quantification of gene expression changes at the level of mRNA has proved to be an interesting tool in environmental approaches [100]. The transcriptomes of *M. spretus* mice captured in areas of high industrial and agricultural pollution such as the Domingo Rubio estuary or the industrial pole of Punta del Sebo (Huelva) have been determined, identifying a set of potential biomarkers of environmental contamination [93]. Likewise, the transcriptional profile of contaminants such as the DDE has been determined in controlled exposure studies in the laboratory [101].

On the other hand, proteomics is of great importance to understand cell homeostasis, to perform quantitative analysis and for the identification of potential biomarkers of *in vivo* toxicity. However, the massive number of proteins and post-translational modifications difficult the analysis. Proteomics can also assist genomic and transcriptomic studies in the efficient sequencing of complete genomes and to explain differences in susceptibility induced by polymorphisms. Nowadays is accepted that alterations in gene expression do not always induce adverse health effects due to post-translational modifications, as phosphorylation and glycosylation of proteins that determine their function, environmental factors or multigenic processes (e.g., aging and disease). For this reason, **environmental proteomics** allows understanding the mode of action of pollutants. Likewise, the use of 1st, 2nd and 3rd generation proteomic methods have been used to compare *M. spretus* mice from different areas within the Doñana National Park (PND) and surroundings, the Estero de Domingo Rubio (EDR) and the Estuary of the Guadalquivir, in order to evaluate the effects of the Aznalcóllar accident and the pesticides used around the PND [102, 103]. The information obtained by this omic can be complemented by **oxidative stress and redox proteomics**. The study of reactive oxygen species (ROS) related to oxidative damage caused by contaminants is a valuable tool in environmental studies [94]. Although most amino acids are sensitive to oxidation, the thiol group of Cys affects them especially [95] so its analysis allows determining the redox state of the thiol groups of proteins [104, 105], so its analysis allows determining the redox state of the thiol groups of proteins.

Environmental metallomics and chemical speciation requires its own methodology, generally based in the combined use of inorganic mass spectrometry (inductively coupled plasma mass spectrometry, ICP-MS) and organic MS, using a previous chromatographic separation step in order to preserve the integrity of the metal in terms of its union and location in the molecule [106]. Our research group have extensive experience in this field, especially in its projection to the environmental studies [2], and the biological response, at the metallomic and metabolomic levels of the *Mus musculus* mice exposed to As [99], Cd [2] and Hg [103]. In addition, studies with free living animals in Doñana National Park and surroundings based on these methodologies have also been performed using the *Mus spretus* mice [11] and the crab *Procambarus clarkii*.

In addition, in 1999, J. Nicholson defines metabonomics as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" [104], and metabolomics as the measurement of all the metabolites in a specified biological sample [104]. Likewise, metabonomics allows understanding the variation in low molecular mass molecules, namely metabolites, which are the last action mechanism of the organisms, but the additional mentioned "-omics" sciences are related to cellular macromolecules. Then, the last step in the omics, directly in connection with the phenotype, **environmental metabolomics** allows obtaining a global view of the metabolic fingerprint of the biological systems exposed to contaminants, providing information at the same time that the interactions between the contaminants with the living organisms [105]. Numerous studies have been carried out on different rodents, and several exposure experiments on the *Mus musculus* laboratory mice can be highlighted. As an example, several studies carried to study of the metabolomic response of the mouse.

M. musculus exposed to inorganic As [99], Cd [95], Se-Cd [2], Hg [99], Hg-Se [28], DDE-Se [27], As, Cd, Se, Hg, deltamethrin + acrolein.

One of the most critical aspects in metabolomics is sample treatment, to extract as many metabolites as possible [10]. The analysis of biofluids for metabolomics in simpler than the extraction of metabolites from tissues and allows obtaining global

information about the state of the organism, but to obtain specific information on a specific organ the direct analysis is mandatory [106].

Finally, metabolomics allows the simultaneous measurement of hundreds of metabolites *in vitro* cell cultures, *in* vivo tissues and even in non-invasive blood and urine biofluids. However, the current drawback of this omic is the standardization of quenching, metabolite extraction procedures as well as the complexity of data analysis and interpretation. Moreover, the influence of factors in the results is important (e.g., age, gender, diet, stress, housing conditions, health status). To overcome these limitations, combination of omics seems to be the best option. However, the temporal space is different in metabolomics and proteomics (i.e., metabolomics gives information about what happens right now, but it can be related with numerous post-translational modifications happened previously). In this sense, it seems that the combination of genomics with metabolomics is easier. Thus, when metabolomics data are interpreted in combination with genomic, transcriptomic and proteomic results, in the so-called systems biology approach, a holistic view of the organism or biological process under investigation can be attained.

6. Concluding remarks

The evaluation of the real impact of a pollutant, and in particular of pesticides can be performed in the different environmental compartments or preferably in mammals to decipher the biological response. In this case, the study can be carried out in free-living animals or in laboratory mice exposed to different pollutants that should be combined to evaluate the biological response of the "chemical cocktail" since they can interact in a synergistic or antagonistic fashion. On the other hand, it is essential to have an adequate analytical methodology that allows the correct monitoring of environmental systems in order to establish their quality, and, when appropriate, the application of corrective measures. Undoubtedly, massive methods "the omics" are among the most efficient current tools.

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Conflict of interest

The authors do not have conflict of interest.

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