Chapter 22

Biological Responses of in vivo Studies to Contaminants: A Contribution to Improve Public Health Knowledge

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53446

1. Introduction

Global climate changes and ecosystems deterioration due to several human activities involving environmental hazards have a great impact on human welfare [1]. These factors added to many other stressors are responsible for emerging diseases worldwide, which represent an important endeavour. For this reason the risk of contaminants on human health is an expanding area of environmental epidemiology. This sub-field of epidemiology addresses, not only the environmental factors affecting the health and illness of populations but also offers public education on environmental issues. Matters such as sources of environmental contaminants, assessment of how exposure to a hazardous chemical may occur, measurement of health effects, and applying appropriate controls are relevant issues of this branh [2]. Surveillance platforms for hazardous environmental factors involving data collection and analyses, and public health promotion through evidence-based approaches are also within the scope of this growing field. Nowadays, attention to chemical and physical factors have gained special care within the scope of environmental epidemiology due to the myriad of pollutants persisting in water bodies, air, crop lands, and other environmental settings.

The example of the adverse effects on humans and other species induced by heavy metals, and metalloids spread within environment [3-12] illustrates the intersection between the environmental and human health issues. Another example, based on epidemiological studies has revealed that pesticides may cause birth deficiencies, and cancer [13].

Latest advances in scientific research and new developments on important environmental and human health topics such as the potential risk to humans from toxic chemicals in the environment were recently communicated [14]. In this matter, research elucidating the cellular and molecular mechanisms by which these environmental agents induce toxicity, mutagenesis, and carcinogenesis, among others were underlined.

Human exposure to environmental contaminants and their potentially harmful impacts on public health was more recently discussed by a panel of international experts on the conference "Human Biomonitoring: Political benefits – scientific challenges", held in Berlin 2010 [15]. In fact, health measures surveys have been implemented worldwide, and the role of human biomonitoring data has been underlined due to important risk assessment, and risk management procedures. Quantitative measure of exposure to environmental chemicals by measuring them or their metabolites in tissues (eg. hair, nails), fluids (e.g., saliva, breast milk, blood, and urine) or exhaled air are indicators of the degree of human exposure to potentially hazardous chemicals. Some widespread health impacts like diabetes, obesity, attention deficit, or hyperactivity are related to chemical exposure, as evidenced by linking human biomonitoring and epidemiological data to health effects [16].

Additionally to this topic, the importance of sentinel animal species for evaluating the potential human health impacts of chemical stressors was previously debated and the possible use of animal data into the human risk assessment process was emphasized [17]. In fact, the scientific literature evidences reports on a wide range of animal sentinels and their relevance as models for epidemiologic studies of human diseases and environmental exposures. For example, domestic and wild animals may be sensitive indicators of environmental hazards and provide an early warning system for public health, corroborating or informing epidemiologic studies in humans [18]. For example, the suitability of pet dogs was focussed since they share the same environment as humans. Additionally, large mammals were pointed in these studies due to the role as top predator species.

Advances in the development of environmental epidemiology are then illustrated by the interrelationships between humans and other forms of life sharing the same environment. In this context, several effects occurring in organisms (eg. mortality, reproductive dysfunction) in a particular ecological niche may alert to potential harmful effects on human health.

Comparison between human and wildlife exposure models revealed similarities in exposure endpoints, chemical stressors (i.e., pesticides and metals), and extent of model validation (for review see) [19].

More recently the topic "Contaminant and Pollutant effects" was discussed in a workshop organised by the Society of Environmental Toxicology and Chemistry (SETAC e Italian Branch), and newly reported [20]. Under this concern, this working group underlined that almost all the contaminants thought to be of concern in large marine vertebrates (eg. pelagic fish, whales) is of great relevance to contaminant-related human diseases. In addition, concerns over newly emerging threats such as perfluorinated compounds and nanomaterials

http://dx.doi.org/10.5772/53446

were debated. Workshop participants also highlighted the role of biomarkers to accomplish this study. To reinforce this idea the relevance of biological responses of some species was mentioned to clarify questions concerning the behaviour of persistent organic pollutants, and emerging contaminants and their impact on humans and the environment [21].This report focussed on the importance of archiving biological and environmental samples (eg. plankton, fish, marine mammals, water, sediment) for monitoring anthropogenic contaminants and a global coordination need in environmental research.

The measurement of the health consequences due to contaminants exposure is a very hard task, since different actors play in this scenario. Factors such as complexes mixtures within the environment (including chemical and biological agents), added to physical parameters (eg. see level rise, heat), and individual factors (eg. species, gender, age, nutritional, and immunological status) take an important part in this evaluation.

Apart from field studies, the use of laboratory animals in biomedical research for the benefit of human health conducted in accordance with ethical procedures, and based on the 3Rs (replacement, reduction, and refinement) remain vital. These reports emphasize the crucial value of research findings on animal species for understanding the extent and burden of health-related problems induced by xenobiotics. In fact, decisions in public health based on these findings contribute for a better formulation of health policy planning and intervention.

Although substantial progress has been devoted to the production, management, and disposal of chemicals, added to the Directives and other regulations aiming to minimise threats, past and present experiences revealed that, it is still a very multifaceted process due to several interacting factors among genetic features and environmental exposures, which deserve much attention.

This chapter is a contribution for the *in vivo* data knowledge on the relevance of both experimental laboratory mice and field studies including wildlife species in which concern to contaminants. Although some exposure models for either wildlife or humans are available following regulatory guidelines focussing on relevant endpoints, some examples, offered as a case study, include, among others, those based on our experiments. For example, the disease potential of some copepods due to their role as vectors of waterborne pathogens of humans is focussed; some fish species used as pollutants indicators are mentioned; finally, some experimental studies conducted on laboratory mice exposed to some contaminants are analysed. Those reports based on interdisciplinary techniques including histopathological, ultrastructural, biochemical, molecular and/or analytical approaches aims to characterize the biological responses to several contaminants within target organs. The last goal intends to integrate these experimental findings for environmental monitoring, and is a contribution for the understanding of human health impacts.

Finally, some issues for a more robust public health-based interdisciplinary research are recommended.

2. Relevance of animal studies for human health

A growing body of research focuses on the impact of chemical contamination on a wide range of animal species among different phyla and habitats. Adverse effects have been described alerting for the risk to humans. In this part, some representative examples are given with special emphasis for zooplankton, and fish. In addition, studies on the toxicity of contaminants on laboratory mice are presented.

Birds have been used to evaluate the presence of heavy metals in some habitats [22]. Some marine gastropod molluscs such as *Nucella lapillus* (L.) are used as bioindicators of tributyltin pollution in the North Atlantic coastlines through an important imposex assessment index [23]. Amphibians can serve as crucial and valuable research models for understanding the ecological effects of persistent contaminants such as mercury amplifying the risk of transfer of accumulated contaminants to higher trophic levels [24]. Amphibians living near uranium mines were used to assess the impacts of the locally produced acidic and metal-rich materials [25]. Copepods have broad geographic ranges, and sustain the world fisheries that nourish and support human populations [26]. They are key sensitive indicators of local and global climate change, and potential vectors of waterborne diseases. Mussels such as *Mytilus galloprovincialis,* and *Mytilus edulis* are key bivalves widely used as sentinel species [27, 28]. Mussels are suitable test species for monitoring persistent pollutants because they are sessile, filter feeders with low rates of metabolic transformation [29].

The biological signals of exposure in the above mentioned species include suitable biomarkers (eg. subcellular, cellular, functional levels) added to pollutant analysis in species. Of course, parameters such as biological cycles must be regarded as for a proper interpretation of data. Another factor to consider when dealing with wild animals is the availability of food once it may have a great influence on the immune system condition of animals [25].

2.1. Role of zooplankton with special reference to copepods in human health

The name plankton is derived from the Greek adjective - *planktos*, meaning "errant", and by extension "wanderer" or "drifter". This is in contrast to nekton organisms that can swim against the ambient flow and control their position (e.g. squid, fish, and marine mammals). Though many planktic or planktonic species are microscopic in size, plankton includes organisms covering a wide range of sizes, including large organisms such as jellyfish.

Plankton are any drifting organisms (animals, plants, or bacteria) that, inhabit the pelagic zone of oceans, seas, or bodies of freshwater. That is, plankton are defined by their ecological niche rather than phylogenetic or taxonomic classification. They provide a crucial source of food to larger, more familiar aquatic organisms such as fish and whales. Freshly hatched fish larvae are also plankton for a few days as long as they cannot swim against currents. Their density and distribution pattern varies horizontally, vertically and seasonally. This variability is mainly due to the availability of light, availability of nutrient. Besides from representing the bottom few levels of a food chain that supports commercially important fisheries, plankton ecosystems play a role in the biogeochemical cycles of many important chemical elements, including the ocean's carbon cycle. In addition, plankton species play im-

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portant role in the disposal of sewage and in the natural purification of polluted water. But, some of them like dinoflagellates, their harmful bloom causes high mortality in the aquatic environment. They also act as indicator of petroleum too [30]. They are responsible for causing various diseases to animals and human beings.

Plankton are primarily divided into broad functional (or trophic level) groups: Phytoplankton (from Greek *phyton*, or plant), autotrophic, prokaryotic or eukaryotic algae that live near the water surface, where there is sufficient light to support photosynthesis. E.g. diatoms, cyanobacteria, dinoflagellates and coccolithophores; Zooplankton (from Greek *zoon*, or animal), small protozoans or metazoans (e.g. crustaceans and other animals) that feed on other plankton; Bacterioplankton, bacteria and archaea, which play an important role in remineralising organic material down the water column.

Plankton are further classified into holoplankton, which spend their entire life cycle as plankton (e.g. most algae, copepods, salps, and some jellyfish) and meroplankton, which lead a planktonic life only for a part of their lives (usually the larval stage), and then develop in to a nektic or sea floor living benthic form (eg: larvae of sea urchins, starfish, crustaceans, marine worms, and most fish.

Plankton are also classified into the following groups, based on the size of these organisms (Table 1).

Plankton inhabits in ponds, lakes, seas and oceans too and their density and distribution pattern varies horizontally, vertically and seasonally. This variability is mainly due to the availability of light, availability of nutrient.

Zooplankton can also act as a disease reservoir. They have been found to house the bacterium *Vibrio cholerae*, which causes cholera, by allowing the cholera vibrio to attach to their chitinous exoskeletons. This symbiotic relationship enhances the bacterium's ability to survive in an aquatic environment, as the exoskeleton provides the bacterium with carbon and nitrogen.

Copepods are known for extensive and varied type of parasitism. In this process they show all degrees of modifications right from slight reduction to complete disappearance. Several marine animals such as anemones, annelids, crustaceans mollusks, tunicates, fishes and even whales and sharks too are being parasitized by the copepods. They can be ectoparasites or endoparasites. Members Lernaeopodidae and Lernaeidae family (Or: Copepoda) are well known ectoparasites. Copepods of Family Caligidae are ectoparasites found on the gills, buccal cavity, and opercular cavity [31].

Copepods are multicellular animals, more abundant than any other groups including insects and nematodes [32]. Though they are mostly inhabit in natural and man-made aquatic systems, they can also inhabit nutrient-rich black oozes of abyssal ocean depths to the nutrient-poor waters of the highest mountain tarns. Some of them are found on canopies of some rain forests, hot springs, leaf-litter, in caves, between sand grains (Figure 1).

They also exhibit symbiotic associations with other animal and plant species. Their density can be as high as 92,000 individuals/L [33].

Copepods form a subclass belonging to the subphylum Crustacea (crustaceans). Copepods are divided into ten orders. Some 13,000 species of copepods are known, and 2,800 of them live in freshwaters [34].

Group	Size Range	Example
Megaplankton	(>20 mm)	Metazoans; e.g. Jellyfish; Ctenophores; Salps (e.g. Cyclosalps, Members of genus Thalia) Pelagic Tunicates (e.g. Sea Tulip, Sea Squirts or Sea Pork); Cephalopodes (e.g. Octopus, squid, Cuttle fish)
Macroplankton	(2–20 mm)	Metazoans; Chaetognaths (eg: Sagitta); Euphausids (e.g.: Antarctic krill), Medusae (e.g. Coelenterates); ctenophores; salps, doliolids (e.g. Doliolum) Tunicata Cephalopoda
Mesoplankton	(0.2 -2 mm)	Metazoans; e.g. copepods; Medusae; Cladocera; Ostracoda; Chaetognaths; Pteropods; Tunicata; Heteropoda
Microplankton	(20–200)	Large eukaryotic protists; most phytoplankton; Protozoa (Foraminifera); ciliates; Rotifera; juvenile metazoans - Crustacea (copepod nauplii)
Nanoplankton	(2–20 μm)	Small eukaryotic protists; Small Diatoms; Small Flagellates; Pyrrophyta; Chrysophyta; Chlorophyta; Xanthophyta
	(0.2–2 μm)	Small eukaryotic protists; bacteria; Chrysophyta

Table 1. Classification of plankton based on the size of organisms [30].

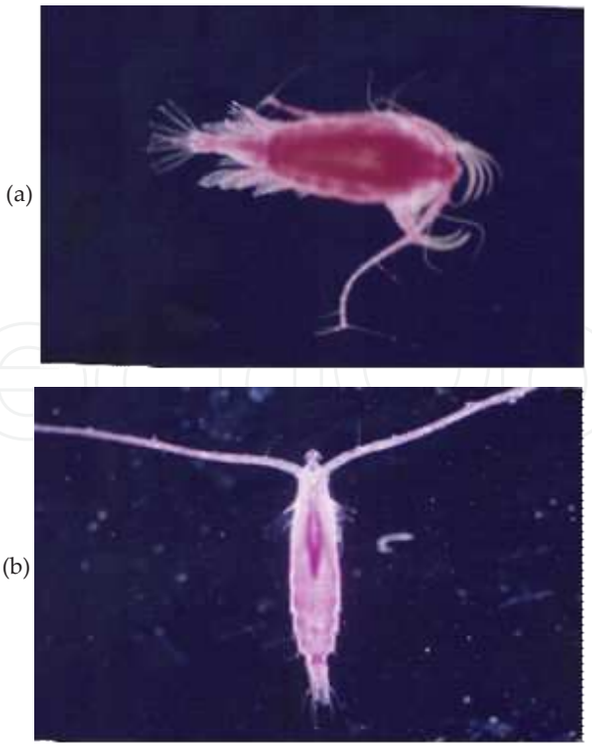

Figure 1. (a) and (b). Representative specimens of copepods: *Euchaeta marina* (a), and *Rhinocalanus cronutus* (b).

http://dx.doi.org/10.5772/53446

Copepods are known to carry pathogenic strains of *Vibrio* and have the potential to play important roles in cholera transmission [35]. This acute bacterial infection of the intestine caused by a few strains of bacterium *Vibrio cholerae* is transmitted through consumption of contaminated water. The bacteria needs chitinous surface of copepods to replicate. In Asia and Africa due to seasonal blooms of phytoplankton, which happen to be the food of the main food source of copepods, increases the amount of cholera-causing bacteria in waterways. When people consume bacteria infected copepod colonized water, they get cholera. The bacteria cause severe watery diarrhea, known as "rice water" diarrhea, and vomiting. Fluid loss can lead to severe dehydration and death within 24 hours of the onset of symptoms if dehydration is not treated. Poor sanitation, mixing up of sewage containing feces from cholera infected patients further contaminates water sources, allowing cholera to continue to propagate.

Apart from this aspect they are vectors of relevant human diseases. Fasciolasis also known as Distomatosis and Liver rot is an important disease caused by trematode helminthes viz., *Fasciola hepatica* (the common liver fluke or sheep live fluke) and *Fasciola gigantica*. This disease belongs to the plant-borne trematode zoonoses. In Europe, the Americas and Oceania only *F. hepatica* is common while in Asia and Africa both species have found in common. Estimated loss by this disease is pegged around US$3.2 billion per annum. As around 2.4 million people are infected ad over 180 million people are at risk, WHO has considered this disease as an emerging human disease. *Fasciola hepatica* is a parasitic flatworm (Cl: Trematoda, Ph.: Platyhelminths), that infects the liver of various mammals, including humans. *F. hepatica* is distributed worldwide, and has been known as an important parasite of sheep and cattle for hundreds of years, causing great economic losses in sheep and cattle. Thus has been the subject of many scientific investigations and may be the best known of any trematode species. They have a wide range of definitive host, which includes many herbivorous mammals, including humans. They have freshwater snails such as *Galba truncatula*, (in which the parasite can reproduce asexually) as an intermediate host for completion of their life cycle. For completion of liver fluke they pass through cercaria and miracidium larval stages, which are also considered as planktonic stages. Though a freshwater snail is required as a intermediate host, for the *F. hepatica* to complete its life cycle, Species in the family Lymnaeidae include: *Austropeplea tomentosa*, *Austropeplea ollula*, *Austropeplea viridis*, *Radix peregra*, *Radix lagotis*, *Radix auriculari*, *Radix natalensis*, *Radix rubiginosa*, *Omphiscola glabra*, *Lymnaea stagnalis*, *Stagnicola fuscus*, *Stagnicola palustris*, *Stagnicola turricul*, *Pseudosuccinea columella*, *Lymnaea viatrix*, *Lymnaea neotropica*, *Fossaria bulimoides*, *Lymnaea cubensis*, *Galba truncatula*, *Lymnaea cousini*, *Lymnaea humilis*, *Lymnaea diaphana*, *Stagnicola caperata* and *Lymnaea occulta* that serve as naturally or experimentally intermediate hosts of *Fasciola hepatica*.

Adult specimens live in bile passages of the liver of many kinds of mammals in general and ruminants in particular. Humans are also occasionally infected. In fact, fascioliasis is one of the major causes of hypereosinophilia in France. The flukes feed on the lining of biliary ducts. Their eggs are passed out of the liver with bile and into the intestine to be voided with feces. If they fall into water where the eggs complete their development and turn into miracidia, which hatch in 9 to 10 days during warm weather. During cold sea-

son it takes longer period. On hatching, miracidia have 24 hours in which find a suitable snail host. Mother sporocysts produce first generation rediae, which in turn produce daughter rediae that develop in snail's digestive gland. From the snail, minute cercariae emerge, which swim through pools of water to pasture and encyst as metacercariae on near-by vegetation. When ruminants feed on this vegetation or when human eat uncooked/semi cooked food prepared out of these plants from the pasture, the metacercariae find their way into ruminants or in human beings. In the liver, due to low pH, these encysted metacercaria start excystement. Later, these parasites break free of the metacercariae and burrows through the duodenum and intestinal lining into the peritoneal cavity. Though the newly excysted juvenile does not feed at this stage. After some days, once they find the liver parenchyma, they start feeding. This immature stage in the liver tissue is the pathogenic stage, causing anaemia and clinical signs sometimes observed in infected animals. The parasite feeds on liver tissue for a period of about six weeks, and later moves into bile duct, where it matures into an adult and begins to produce eggs. Under mild infection, these organisms can produce up to 25,000 eggs per day per fluke and can produce and deposit up to 500,000 eggs onto a pasture by a single sheep.

Trypanosomiasis also called as Chagas disease is caused in several vertebrates like horses, buffalo, dogs, cats and also human beings by parasitic protozoan trypanosomes of the genus *Trypanosoma*. It is reported that around 500,000 men, women, and children in 36 countries of sub-Saharan Africa suffer from human African trypanosomiasis and around 21,000 die due to this disease. The main casual agents are T*rypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*.

Basically there are two types of trypanosome species viz. salivarian species and the stercorarian species trypanosoma exists. Stercorarian trypanosomes infect the insects like triatomid kissing bug, develop in its posterior gut and infective organisms are released into the faeces and deposited on the skin of the host. The organism then penetrates and can disseminate throughout the body. Insects become infected when taking a blood meal. While the second type, namely Salivarian trypanosomes develop in the anterior gut of insects, most importantly the Tsetse fly and infective organisms are inoculated into the host by the insect bite before it feeds.

As trypanosomes progress through their life cycle they undergo a series of morphological changes as is typical of trypanosomatids. The life cycle often consists of the trypomastigote form in the vertebrate host and the trypomastigote or promastigote form in the gut of the invertebrate host. Intracellular lifecycle stages are normally found in the amastigote form. The trypomastigote morphology is unique to species in the genus *Trypanosoma*.

The role of copepods as intermediate hosts of the fish parasite *Diphyllobothrium latum* is well known [36]. The life cycle of this tapeworm include copepod, and fish as first and second hosts, respectively. Human consumers of raw or lightly processed fish are final hosts. The debilitating disease, dracunculosis is vectored by freshwater copepods such as *Mesocyclops kieferi*, *M. aspericornis*, *Thermocyclops incisus*, *T. inopinus* and *T. oblongatus* of the Guinea worm, *Dracunculus mediensis* [35]. Though stomach digestive juices destroy the copepods,

http://dx.doi.org/10.5772/53446

the larvae of the Guinea worm survive and penetrate the stomach or small intestinal wall, migrating to the subcutaneous tissue of the abdomen and thorax.

Other adverse impacts on human health of copepods include their potential as important allergens [37].

2.2. Relevance of edible fish

This section deals, mainly, with the relevance on contaminants on fish, due to the relationship between the environment and human populations through diet.

Contaminants are among the anthropogenic stresses with negative impact on water quality, thus contributing for general decline on several fish species which economical value is well recognised [38]. Moreover, hazardous chemicals alter the chemical composition of water, thus may render them susceptible to infectious diseases, being a source for several zoonotic diseases agents.

Fish is considered as one of the best indicators of heavy metal contamination in coastal milieu [39]. A number of fish species has been used as relevant bio-indicators in monitoring programs around the world. For example, skipjack tuna (*Katsuwonus pelamis*) has been used for global monitoring of wide range pollutants (eg. PCBs and organochlorine pesticides, polybrominated diphenyl ethers, polychlorinated dibenzo-p-dioxins, furans, and coplanar polychlorinated biphenyls [40-43]. Red mullets (*Mullus barbatus*) were used as sentinel organisms from anthropogenic impacted areas at Mediterranean Sea [44]. The Atlantic cod, *Gadus morhua* has been used in the Barents Sea sub-Arctic location for PAHs and metals monitoring [28]. *Anguilla anguilla*, *Platichthys flesus* and *Dicentrarchus labrax* are other bioindicadors of contaminants in different geographical areas [45-48].

Bottom feeders such as mullets, and flounders concentrate contaminants to a higher degree than other species.

To evaluate the biological responses of contaminants on fish a wide range of techniques have been used namely histopathology, and biochemistry of enzymes. Biomarkers such as DNA integrity and detoxification enzyme status in fish tissues have also frequently been used. Other biomarkers of aquatic pollution including fish phagocytes-induced ROS, peroxidative damage, and oxidative stress were reported on the European eel *Anguilla anguilla* [49, 50]. A battery of biomarkers together with gonad histology was used to characterize the responses of red mullets (*Mullus barbatus*) to anthropogenic pollutants [44].

The effects of some stressors were evaluated on *Platichthys flesus*, using several biomarkers [46]. Different patterns of response, namely enzymatic, genotoxic, and cytotoxic were observed on fish from impacted areas, underlying the role of reliable biomarkers for future biomonitoring studies. Other studies reported on biological responses of this flatfish to chemical stress including gene expression, genotoxicity, cholinesterase, and growth rate [51].

Novel biomarkers at molecular level, such as alterations in the expression of *xpf* gene and some of the genes found by SSH, such as HGFA were described in *Dicentrarchus labrax* and *Liza aurata* exposed to environmental contaminants [52].

Adverse changes on liver of *Poecilia vivipara* assessed semi-quantitatively using the (Histopathological Alterations Index – HAI) were described and the possibility of relationship to pesticides, heavy metals, sewage and others factors were postulated [53]. Other potential markers on environmental pollution were illustrated by analyse of matrix metalloproteinases on fish bile from *Mugil liza* and *Tilapia rendalli* [54].

High concentration values of heavy metals exceeding the permissive levels and the allowable maximum concentrations of these pollutants on organs of edible fish were reported in different species worldwide, suggesting that fishes could cause serious problems to human health, due to bioaccumulation over time; for example high levels of the most hazardous metals on muscular tissue of *Oreochromis niloticus* were reported (1.315 Cd 2.053 Pb 1.159 Hg mg/kg) representing an hazard at human health point of view [55]. Among nine commercially important species, the concentration of Mn, Fe, and Pb in a few species exceeded the WHO guideline values for safe human consumption [39]. For example muscle samples of *Lepturacanthus savala* displayed maximum levels of Pb (2.29 μg g−1). Similarly, chemical analyzes on four species (*Sarda sarda, Mulus barbatus ponticus, Trachurus trachurus* and *Merlangius merlangus* using flame and graphite furnace atomic absorption spectrometry revealed acceptable values of trace element levels. However, lead and cadmium (e.g. 0.28 ± 0.03 μg/g Pb and 0.35 ± 0.04 μg/g Cd/ *Sarda sarda*) in fish samples were higher than the recommended legal limits [56].

High mercury levels in organs of *Liza aurata* inhabiting a contaminated estuary were reported, and human risk associated to the ingestion of fish was not excluded [57].

Other reports mention lower concentration of some pollutants on fish tissues, namely muscle, being suitable for human diet. However, the influence of chemical interactions must be considered. For example heavy metal concentrations in different tissues of *Labeo rohita* and *Ctenopharyngodon idella* of Upper Lake of Bhopal were within the recommended limit values for fish consumption [58]. Although the results of this study confirm the safety for human health, these authors alert for the need of further preventive measures, since it is quite evident that there was accumulation of heavy metals in fish tissues.

Studies on heavy metals and metalloid (mercury, cadmium, lead, and arsenic) levels in the muscles of three relevant economically pelagic species (*Sardine pilchardus*, *Scomber japonicus* and *Trachurus trachurus*) from the Northeast and Eastern Central Atlantic Ocean revealed different patterns of contamination according to feeding behaviour [59]. Values of As>Pb>Hg>Cd for sardine, and As>Hg>Pb>Cd for chub mackerel and horse mackerel were reported. This elegant survey also estimated the potential public health risks via consumption of the mentioned species. From those studies it was concluded the safety for human consumption in terms of the amounts of cadmium and lead although moderate intake was recommended due to possible health risks derived from arsenic and mercury.

http://dx.doi.org/10.5772/53446

Another survey, aiming to evaluate potential risk–benefit of fish consumption was conducted using 24 common fish species collected from Chinese markets on 2007 [60]. Nutritional value and contaminants levels (DDT, PCB_7, arsenic and cadmium) were evaluated. Although mercury concentration in common carp exceeded the upper limit of the Chinese national standard these studies indicated that fish, particularly marine oily fish can be regularly consumed to achieve optimal nutritional benefits, without causing significant contaminant related health risks. Nevertheless, potential health warning was referred for people consuming large amounts of fish, namely wild fish.

Some physiological alterations such as, breathing, gastrointestinal, and skin disorders were reported as associated to individuals consuming As-contaminated fish daily and the potential risk of arsenicosis among poor people was underlined [61].

Some of the examples above mentioned illustrate the significance of pollutants release into the environment on edible fish species which may represent a warning for public health. Therefore, these findings may lead to public health interventions and policy initiatives for safeguarding human health.

2.3. Experimental studies with laboratory mice

The following section presents some examples of our experimental studies conducted with laboratory mice, aiming to illustrate the effects of some hazardous compounds at different levels of biological response (cellular, tissue, and/or physiological). Efforts aiming a cross-disciplinary interaction were done and several techniques were used, such as histology, transmission electron microscopy, and flow cytometry. These approaches complemented with chemical analysis of the toxicants or its metabolits on tissues exemplify the relationship between contamination, and injury.

Apart from our experience using this model the scientific evidence of adverse health effects from ubiquitous anthropogenic pollutants (eg. metals, metaloids, and pesticides) on both animal species and humans has been largely documented, thus, contributing to identify potential harmful chemicals for human health.

Some environmental toxicants have been shown to exhibit deleterious effects on testis, namely spermatogenesis, and fertility.

There is growing evidence that lead ($PbCl_2$) and cadmium compounds ($CdCl_2$) adversely affects spermatogenesis and fertility parameters of mice indicated by severe degenerative changes on seminiferous tubules, and poor quality of semen [62, 63]. Histopathological studies were also conducted in order to explore the extension of damage. In addition, flow cytometry (FCM) studies were performed using buffered formalin fixed and paraffin embedded paraffin samples of testicular tissue from mice exposed to cadmium and lead chloride per se, in order to establish the ploidy level of germ cells. Significant alterations in germ cell percentages in mice exposed to cadmium were detected by FCM, supporting the histopathological data [62]. However, no alterations in the percentages of testicular germ cells detected by FCM were evident in animals exposed to lead chloride, excepting of an increase in the percentage of cells in S phase.

Further reports, by using several biomarkers at subcellular level of sperm physiology such as DNA fragmentation, and chromatin integrity, assessed by the terminal deoxylnucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick-end labelling (TUNEL), and sperm chromatin structure assays (SCSA), respectively, coupled to other techniques for evaluation of sperm function authors demonstrated that lead chloride affects physiological parameters such as motility, morphology, and acrosome status, although no significant genotoxic effects were noted [63]. In fact, sperm DNA is in general resistant due to its highly compacted nature. Those reports also evidenced the role of flow cytometry as a powerful tool for quantitative analyses of different cell types and an insight on cell cycle status, added to the measurement of DNA content of cell subpopulations in the testis.

More recently the genotoxic effects induced by cadmium were reported using a panel of suitable microsatellites as markers of genetic instability [64].

The effects on testis after co-exposure to lead and cadmium were also described on mice through a histological approach [65]: seminiferous epithelium degeneration, exfoliation of germ cells into the lumen, distorted morphology of tubules was accompanied by atrophy. Work in progress in our laboratory demonstrated adverse effects of this mixture on other target organs such as kidney, spleen, and liver, comparing with respective controls (Figures 2 and 3). Degenerative aspects were noted on renal cortical area; splenic sections denoted cell loosening and numerous macrophages; hepatic parenchyma displays several haemorrhagic foci. Overall, the histopathological study revealed several adverse changes pointing obviously for dysfunctions.

Another illustration of a toxicant targeting the male reproductive function of mice was the research conducted with sodium arsenite [66]. Awareness was directed to spermatogenesis, a multipart process involving delicate cells such as germ cells, often targets of a wide range of contaminants. Impairment of spermatogenesis was found on testis sections, complemented with biochemical parameters. Those issues have been established as suitable in the evaluation of physiological disorders in mice.

Further studies aiming to explore and characterize possible recovery of sperm morphology and functional parameters after withdrawal of the toxicant revealed several changes at ultrastructural level, namely irregular pattern of chromatin, and altered acrosome [67]. In addition, a molecular approach demonstrated high DNA fragmentation index revealing abnormal chromatin structure. In this work, as in present studies on this topic, transmission electron microscopy studies were pertinent to identify fine changes on cell organelles (Figure 4).

Work in progress also demonstrated the effects of sodium arsenite on splenic structure of dosed animals (Figure 5). The degree of lesions was irregular: some areas of the spleen evidenced reduced cellular concentration (Figure 5a) and disruption (Figure 5b), respectively, within white pulp comparing with controls (data not shown). In addition, an increase of megakaryocytes was observed, probably due to the required phagocytic activity.

Histopathology coupled with ultrastructural studies were sensitive tools for the detection of adverse effects of sodium arsenite on target organs (eg. testis) allowing information on the nature of the lesions, and its eventual recovery.

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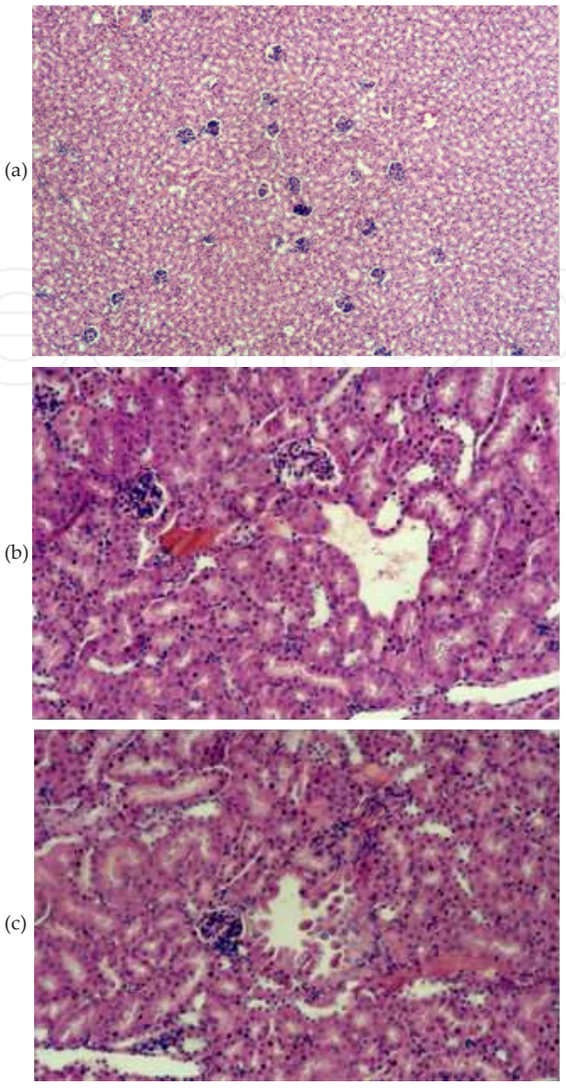

Figure 2. Representative histological section from kidneys of controls where regular pattern of organization is observed (a), and lead and cadmium co-exposure on mice ((b), and (c)). Figures (b), and figure (c) display haemorrhagic focus, and strong dilation of intertubular spaces. Cell detachment is evident. Haematoxylin & eosin staining (HE). Original magnification: Figure (b) – 40x; Figure (c), and 5 – 100x.

The relevance of toxicity of some chromium compounds, namely hexavalent chromium generated by production industries such as leather tanning, and chrome plating, were investigated on mice [68-70]. In these studies Cr(VI), a proved strong carcinogenic, was investigated in vivo in order to evaluate Cr(VI), and Cr(V) reduction effects on the target organs such as testis, liver, and kidneys. For example it was demonstrated that Cr(V), in the form of $[CrV\text{-}BT]^{2-}$ is a male reprotoxicant, causing several histological and ultrastructural changes in mice spermatogenesis [68]. One of the most representative lesions was the loss of acrosome sperm integrity, as demonstrated by electron microscopy studies. Adverse effects were also observed on cauda epididymis, namely epithelial vacuolation. Altogether, these lesions confirm the potent toxicity of this compound.

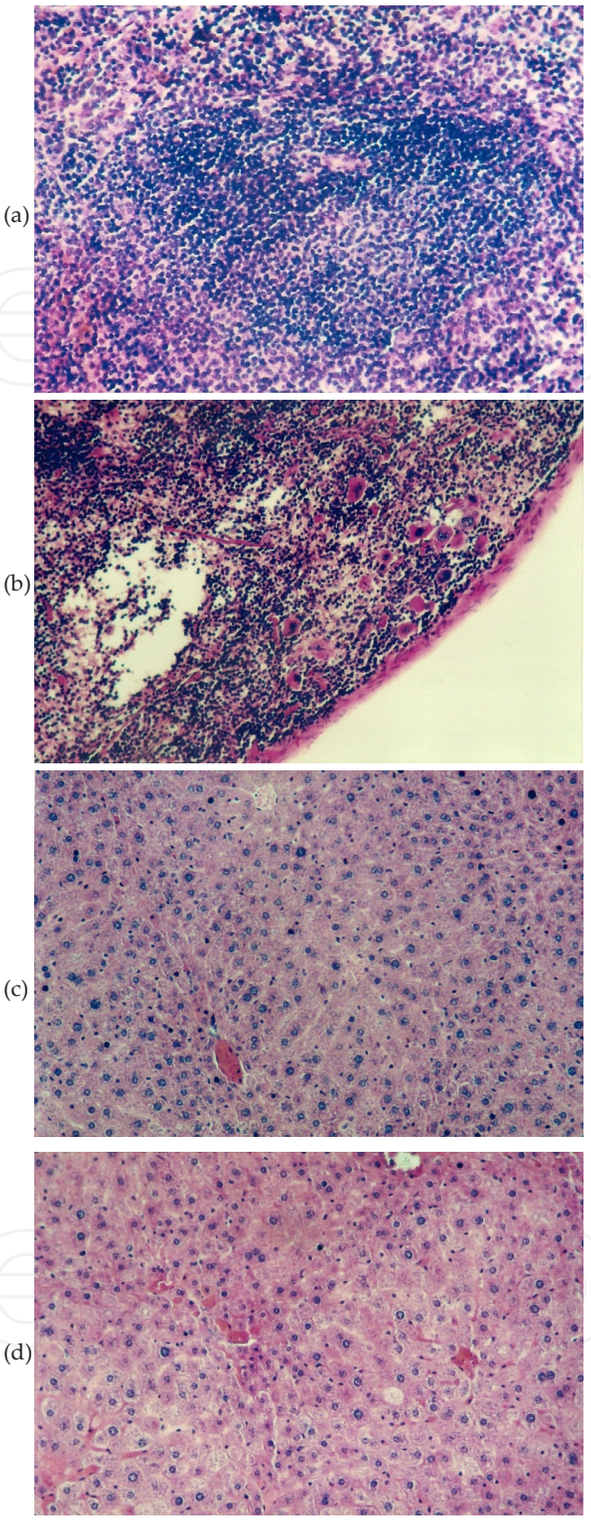

Figure 3. Representative histological sections from spleen, and liver from controls (Figures a,c), and cadmium-lead exposed mice (Figures b,d). A great number of megakaryocytes are evident in spleen (Figure b); WP – White pulp. Several hemorrhagic areas are seen in hepatic parenchyma (Figure d). Original magnification: Figures a-d – 100x; haematoxylin & eosin staining.

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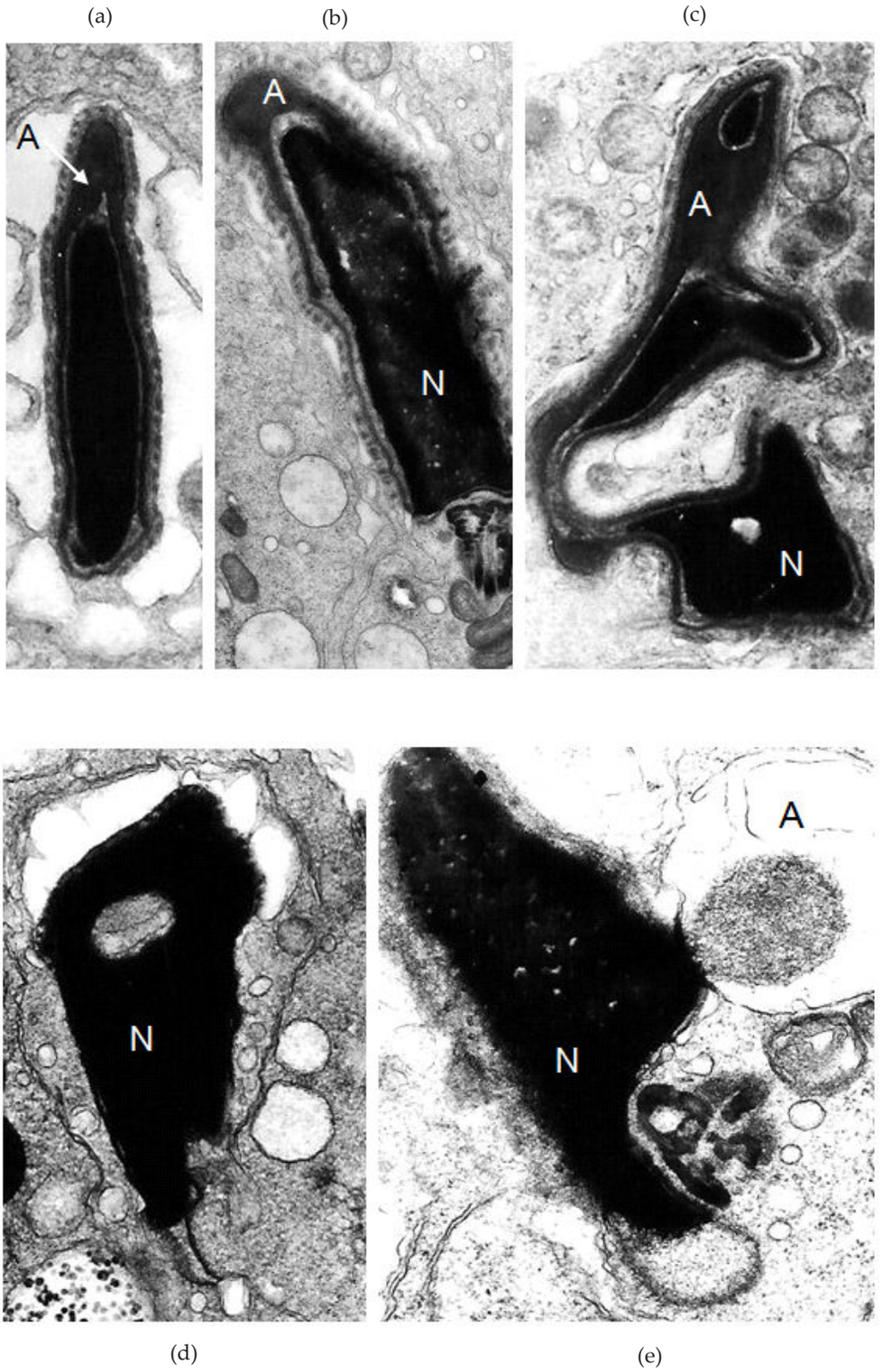

Figure 4. Transmission electron micrographs from spermatozoa and late spermatids in control (a) and sodium arsenite exposed animals during seven days where some morphological irregularities are seen (b-e). N – nucleus; A – acrosome; double staining with uranyl acetate and lead citrate. Original magnification : Figures a -c – x6,700; Figure d – x10,000 ; Figure e – x14,000.

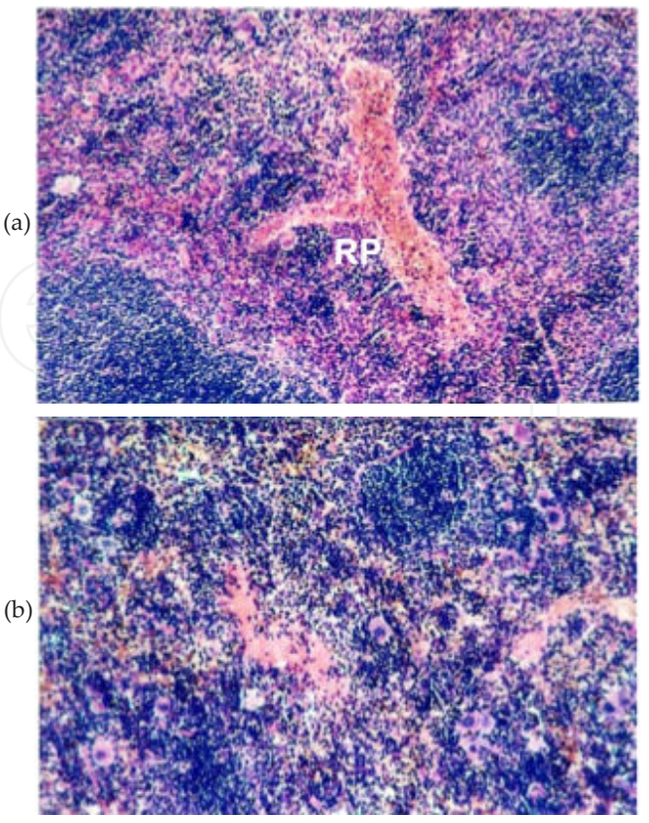

Figure 5. Representative sections of spleen from sodium arsenite dosed group, displaying injury of red pulp (RP) (a), and altered morphology pattern of white pulp (b). Original magnification Figure 5 –x100; haematoxylin & eosin staining.

In a subsequent study the reproductive toxicity on testis and sperm cell's function was evaluated on mice exposed to potassium chromate through a wide range of approaches for a more comprehensive analysis of its effects [70]. Chromium contents on mice testes were determined by inductively coupled plasma mass spectrometry (ICP-MS), and higher levels of chromium were found on K_2CrO_4 exposed group. Histology data of testis were supported by the analysis of testicular cellular subpopulations by flow cytometry. Multiple abnormalities were noted on sperm cells after one cycle of spermatogenic process, such as decreased motility, and percentage of cells with intact acrosome, revealing a premature acrosome reaction. Although no DNA damage on sperm cells assayed by SCSA was observed, altogether those results underlined the reprotoxic effects of hexavalent chromium compounds. Although no histopathological changes on testis or epididymis were noted, a reduction in seminiferous tubules diameter occurred on exposed animals, as determined by software based on deformable models (Snakes) as previously reported [71]. These authors have demonstrated the suitability of this method for evaluation of seminiferous tubules diameter in mice exposed to chromium compounds, one of the relevant parameters for testis damage. Also, the functional properties of Sertoli cells from mice exposed to Cr(V) were investigated using ultrastructural tracer techniques [72]. Horseradish peroxidase added in vitro to the medium was used to follow the route of macromolecules. Seminiferous tubules were then placed into

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this medium, and the marker penetrated freely on the blood-testis barrier compared to controls, evidencing the toxicity induced by the reduction of Cr(VI) compounds.

Other organs such as kidneys were also affected by hexavalent chromium [69].

Similar approaches were conducted to clarify the nephrotoxicity of chromium copper arsenate (CCA), and its constituents *per se* using mice as models [73-75]. Although this mixture (CCA type C - 34.0% As_2O_5, 47.5% CrO_3 and 18.5% CuO, w/w) was broadly used in the past, as a wood preservative, elevate levels of residues as arsenic still remain in the environment posing a great hazard for public health. For example, CCA, arsenic pentoxide, and chromium trioxide were studied per se on kidneys, based on histopathology, and histochemistry. Correlating histology, and histochemistry with the chromium and arsenic analyses (ICP-MS and GFAAS) on kidney, the synergetic effect of the components (pentavalent arsenic and hexavalent chromium) within the mixture conducted to acute tubular necrosis. The histochemistry assay confirmed the presence of carbohydrate, and proteins filling the tubular lumen; the degeneration of epithelial cells (both in cortex and medulla) was also noted. In addition, higher values of arsenic in CCA-exposed group when noted to those submitted to As_2O_5 one of the components of CCA.

Some of the abovementioned studies based case-by-case underline the contribution of animal research for the understanding of the adverse effects of harmful chemicals on human health.

3. Conclusion and future perspectives

Globally, the field of environmental epidemiology has gained substantial attention in current days due to the adverse effects on human health induced by the myriad of pollutants persisting in air, oceans and freshwater, and agriculture soils. In addition, natural resources which safeguard and support human life are also affected by contaminants. A growing body of research focuses on how the environment can damage human life, through the understanding of toxicity signs in other animal species among different taxa. In this concern, a wide range of biological responses were described (eg. reduction of the population, changes in reproductive pattern, and loss of some species). Surveillance programs have been conducted around the world using key species for contaminants monitoring. These studies showed that accumulation and biomagnification of current hazardous chemicals occurs in many species.

Zooplankton can very well act as bio indicators for probable and possible spread of diseases. Breaking the link between such kind of zooplankton and the hosts will result in non-completion of the life cycle of several such disease causing agents which are threatening the human kind. This will also help in having a healthy world.

Other relevant aspect to report is the overall benefits on human health populations due to regular fish consumption. Trials conducted worldwide based on the nutrients value suggests that fish intake based on policy recommendation lowers the risk of some diseases. Sev-

eral international regulatory agencies such as the Codex Alimentarius Commission, and European Commission, Council and Parliament and Food and Agriculture Organization/ World Health Organization conventioned maximum limits for numerous contaminants on edible fish.

On the other hand, the accumulation of harmful residues in fish fillets, even below the maximum limit, may alert for future surveillance. A comprehensive risk–benefit evaluation of fish intake is essential and consumption advisories for specific populations such as pregnant women, and children are required.

The last part of this chapter outlines the contribution of laboratory studies using mice for the knowledge of biological mechanisms and toxicity of several hazardous chemicals. The use of laboratory rodents in biomedical research still remains useful to identify potential responses of harmful chemicals under controlled conditions. However, ethics in animal care and procedures must be considered.

The examples mentioned in this chapter highlight that changes occurring in lower levels of biological organization such as copepods, fish, and mammals, added to the environment surrounding affecting humans, may predict, or may alert to potential harmful effects on human health highlighting the role of environmental epidemiology as an emergent discipline. In fact, monitoring health problems within fauna, and health hazards may contribute for searching new solutions, thus improving the quality of live. An integrated monitoring of species from different taxa with trophic relevance permit a more comprehensive outlook of environmental health problems, and is an important step forward to protect wildlife and human health. However, these approaches comparing effects among different species that might occurs in humans as well, needs a special attention in extrapolating result in animals to humans.

In conclusion, this chapter clearly shows that, the contribution of the need for further local, and global multi-and inter-disciplinary research involving critical representative trophic chain species within impacted areas, since they offer important data. The valuable information of human biomonitoring combined with watchfulness on different species and humans sharing the same environment is needed. A continued focus to promote coordination of biobanks, and data harmonisation is encouraged aiming to formulate public health strategies in the future. In addition, new technologies including analytical methods for detection of contaminants, and multiplicity of biomarkers at different biological levels of organization represent decisive advances in this field.

Acknowledgements

This work was funded by the Research Centre on Ceramic and Composite Materials (CICECO) from Aveiro University (Portugal). Pest-C/CTM/LA0011/2011- FCT is acknowledged. Thanks to Mr. Aldiro Pereira for preparing photographs. Thanks to Mr. Aldiro Pereira for preparing photographs.

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